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From Knowledge to Wisdom

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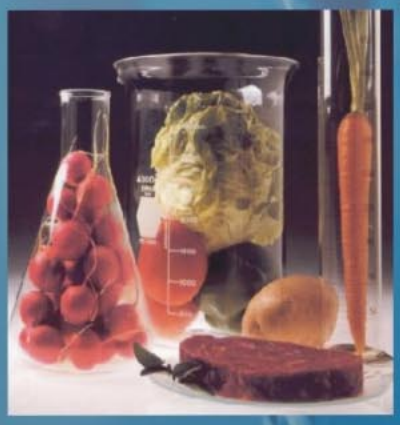

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Inference of Zn in Enormity of the Public Health

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Abstract: Zinc (Zn) deficiencies are currently thought to cause chronic metabolic derangement leading to or exacerbating immune deficiency, gastrointestinal problems, endocrine disorders, neurologic dysfunction, cancer, accelerated aging, degenerative disease, and more. Zn deficiency caused by malnutrition and foods with low bioavailability, aging, certain diseases, or deregulated homeostasis is a far more common risk to human health than intoxication. Higher dose of Zn should be limited to short-term use because of an increased risk of gastrointestinal adverse effects, copper deficiency, anemia, and genitourinary complications. This review has detonated the literature on the spectrum of health effects of Zn status, ranging from symptoms of Zn deficiency to excess exposure.

Key words: Zn, dietary requirements, deficiency, toxicity.

1. Introduction

Zinc (Zn) is the second most abundantly distributed trace element in the body after iron. Because of its nature as a transitional element in the periodic table, Zn possesses certain chemical properties that make it especially useful and important in biological systems. Specifically, Zn is able to constitute strong, but readily exchangeable and flexible, complexes with organic molecules, thereby enabling it to modify the three-dimensional structure of nucleic acids, specific proteins, and cellular membranes and influence the catalytic properties of many enzyme systems and intracellular signaling. Zn is associated with more than 50 distinct metalloenzymes, which have participated in all aspects of intermediary metabolism, transmission, and regulation of the expression of genetic information, storage, synthesis, and action of peptide hormones and structural maintenance of chromatin and biomembranes. Zn is thus needed for growth and development, protein and DNA synthesis, neuro-sensory functions, cell-mediated immunity, thyroid, and bone metabolism. It is normally obtained from red meat and other animal proteins, which not only have high Zn content but the Zn are bounded to ligands that facilitate its absorption [1]. Other sources of Zn are sea foods, dairy foods, cereals and nuts [2]. Zn status of humans for persons with fully functional homeostatic mechanisms is generally determined by the quality and quantity of Zn in the diet and the physiologic condition of the individual. Long-term marginal intakes of Zn increase the risk and severity of a variety of infections, restrict physical growth, skin lesions and impaired wound healing, hypogonadism, anemia, diarrhea, anorexia, mental retardation, and impaired visual and immunological function [3]. Immunosenescence describes the age-related changes in immune function that lead to increased susceptibility to infectious diseases, autoimmunity and cancer. Global recognition of the importance of Zn nutrition in public health has been expanded dramatically in recent years, and more experience has been accumulated on the design and implementation of Zn intervention programs. Therefore, the study was carried out to review Zn physiology, pathophysiology, the importance of Zn deficiency and toxicity.

2. Zn Homeostasis

The human body contains 2-3 g Zn, and nearly 90%

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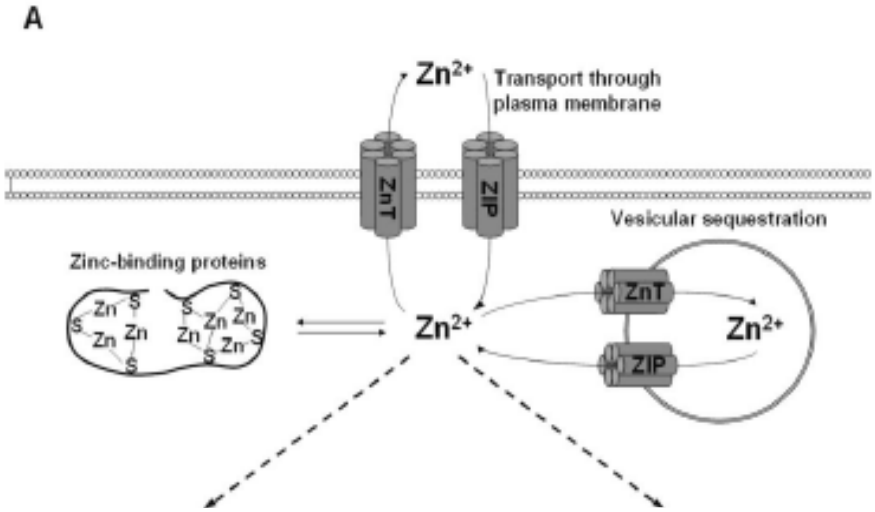
is found in muscle and bone [4]. Other organs containing estimable concentrations of Zn include prostate, liver, the gastrointestinal tract, kidney, skin, lung, brain, heart and pancreas [5-7]. Oral uptake of Zn leads to absorption throughout the small intestine and distribution subsequently occurs via the serum, where it predominately exists to bind to several proteins such as albumin, α-microglobulin and transferring [8]. On the cellular level, 30%-40% of Zn is localized in the nucleus, 50% in the cytosol and the remaining part is associated with membranes [9]. Cellular Zn underlies an efficient homeostatic control that avoids accumulation of Zn in excess (Fig. 1).

Cellular Zn homeostasis is mediated by three main mechanisms. First, transport through the plasma membrane by importers from the Zip-family, and export proteins from the ZnT-family; Second, by Zn -binding proteins such as metallothionein; Third, by transporter-mediated sequestration into intracellular organelles, including endoplasmic reticulum, Golgi and lysosomes. Tight control of Zn homeostasis is required for maintenance of cellular viability, whereas deregulation leads to cell death.

Free and loosely bound Zn ions are bound by the apo-protein thionein (Tred), to form metallothionein (MT). Elevated levels of free Zn ions can bind to Zn finger structures of the metal-regulatory transcription factor (MTF)-1, thus inducing the expression of thionein. Additionally, oxidation of thiols by reactive oxygen (ROS) or nitrogen (RNS) species triggers the formation of the oxidized protein thionin (Tox) with concomitant release of Zn.

The cellular homeostasis of Zn is mediated by two protein families: the Zn-importer (Zip; Zrt-, Irt-like proteins) family, containing 14 proteins that transport Zn into the cytosol, and the Zn transporter (ZnT) family, comprising 10 proteins transporting Zn out of the cytosol. The same transporter families also regulate the intracellular distribution of Zn into the endoplasmic reticulum, mitochondria and Golgi. In addition, many mammalian cell types also contain membrane-bound vesicular structures, so-called zincosomes. These vesicles sequester high amounts of Zn and release it upon stimulation, e.g., with growth factors [10, 11].



Maintenance of cellular vitality
Catalytic, co-catalytic and structural functions
Signal transduction
Protection from oxidative stress
Inhibition of caspases
↑Bci-2/bax ratio

Cell death
↓Bci-2/Bax ratio and release of cytocrome C
Activation of proapoptotic molecules like p38
Activation of potassium channels
Inhabitation of energy metabolism
Induction of oxidative stress

Fig. 1 Cellular Zn homeostasis and its impact on cytotoxicity.

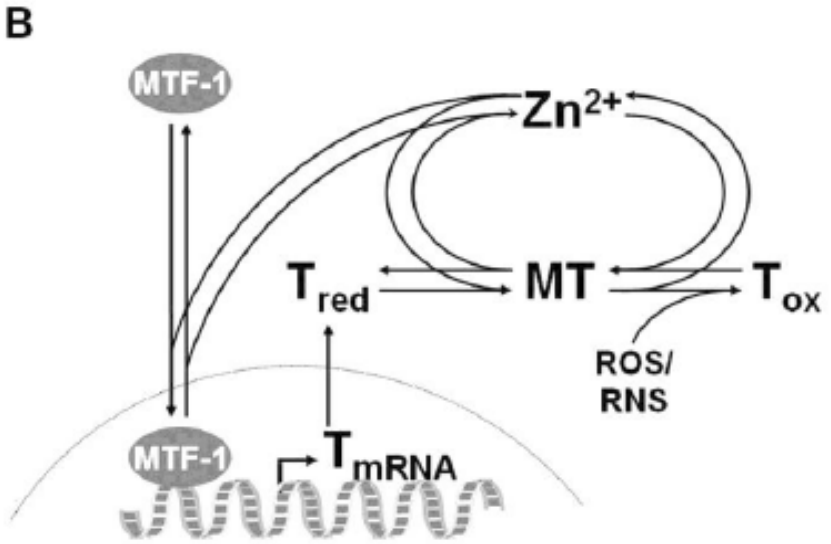


Fig. 2 A particular role in intracellular Zn homeostasis is played by the metallothionein/thionein-system.

Finally, metallothioneins (MTs) play a significant role in Zn homeostasis by complexing up to 20% of intracellular Zn (Fig. 2) [12, 13]. MTs are ubiquitous proteins, characterized by a low-molecular weight of 6-7 kDa, high cysteine content, and their ability to complex metal ions. One MT molecule can bind up to seven Zn ions. Through different affinities of the metal ion binding sites, it can act as a cellular Zn buffer over several orders of magnitude [14]. Dynamic regulation of cellular Zn by MT results from the synthesis of the apo-form thionein (T) in response to elevated intracellular Zn levels by triggering the metal response element-binding transcription factor (MTF)-1 [15].

3. Zn Dietary Requirements

The diet is the primary source of Zn, with drinking water supplying only a small fraction of requirements [16]. As the ability of the body to store Zn is limited, animals require a constant supply to maintain homeostasis. Dietary sources high in bioavailable Zn include shellfish (especially oysters), red meat and liver, poultry (dark meat more than light meat), eggs, dairy products and fish (Table 1) [17, 18].

Zn is present in seeds, nuts, legumes, cereals and soy-based products, but is less bioavailable because these foods contain high levels of phytates (organic phosphates such as inositol hexa- and penta-phosphates) that chelate dietary Zn and iron, making them less available for absorption. Provisional dietary requirements for Zn in relation to estimates of retention, losses, and availability are shown in Table 2 [19]. Zn bioavailability may be affected by the type of Zn salt chosen for supplementation, and it is reduced by dietary calcium, phosphorus, oxalate, copper, magnesium and excess iron [20, 21]. In some studies, Zn that is complexed with amino acids (e.g., histidine, methionine, glutamate, glycine) or other low-molecular-weight organic ligands (e.g., citrate, ascorbate, picolinate, proprionate) appears to be more bioavailable than free or inorganic Zn, and may be less affected by dieary calcium or phytate [22-25]. In humans, vegetarian diets or the avoidance of red meat are risk factors for the development of Zn deficiency [26].

It is difficult to precisely determine the requirement of Zn in various populations, in as much as many dietary factors affect the bioavailability of Zn and environmental and physiological factors alter the requirements of Zn in different age groups. Nonetheless, the current US guidelines for RDAs of Zn (Table 3) appear to be reasonable [27].

Table 1 Zn content of common household portions of selected foods [18].

Food	Portions	Zn (mg)
Fish, light poultry meat, shellfish (except crab and oyster)	3 oz	< 2.0
Poultry liver, dark chicken meat	3 oz	2.0/3.0
Pork, veal, crab, dark turkey meat, 3 oz 3.0/4.0 ground beef, 77% lean	3 oz	3.0/4.0
Beef liver, beef	3 oz	4.0/5.0
Oyster	3 oz	> 5.0
Egg, whole	1	0.5
Peanut butter	2 Tbsp	0.9
Mature dried beans, lentils, chickpeas, split peas (boiled, drained)	1/2 cup	0.9/1.0
Cow peas, black eyed peas (boiled, drained)	1/2 cup	1.5
Milk: whole, fluid	1 cup	0.9
Canned, evaporated	1/2 cup	1.0
Dried, nonfat, instant	1/3 cup	1.0
Ice cream	1 1/2 cup	1.0
Cheddar cheese	3 slices (1 1/2 oz)	1.6
Cooked oatmeal	1 cup	1.2
Cooked whole wheat cereal	1 cup	1.2
Wheat flakes	1 oz	0.6
Bran flakes, 40%	1 oz	1.0
Wheat germ toasted	1 Tbsp	0.9
Corn flakes	1 oz	0.08
Cooked corn meal	1 cup	0.3
White wheat bread	1 slice	0.2
Whole wheat bread	1 slice	0.5
Cooked brown rice, hot	1 cup	1.2
Cooked white rice, hot	1 cup	0.8
Precooked white rice, hot	1 cup	0.4

Table 2 Provisional dietary requirements for Zn in relation to estimates of retention, losses and availability [19].

	Peak daily retention (mg)	Urinary excretion (mg)	Sweet excretion (mg)	Total required (mg)	Milligram necessary in daily diet if content is available		
					10%	20%	40%
Infants							
0-4 months	0.35	0.4	0.5	1.25	12.5	6.3	3.1
5-12 months	0.2	0.4	0.5	1.1	11.0	5.5	2.8
Males							
1-10 years	0.2	0.4	1	1.6	16	8.0	4.0
11-17 years	0.8	0.5	1.5	2.8	28	14.0	7.0
18+ years	0.2	0.5	1.5	2.2	22	10.0	5.5
Females							
1-9	0.15	0.4	1.0	1.55	15.5	7.8	3.9
10-13	0.65	0.5	1.5	2.65	26.5	13.3	6.6
14-16	0.2	0.5	1.5	2.22	22.0	11.0	5.5
17+ years	0.2	0.5	1.5	2.22	22.0	11.0	5.5
Pregnant women							
0-20 weeks	0.55	0.5	1.5	2.55	25.5	12.8	6.4
20-30 weeks	0.9	0.5	1.5	2.9	29.0	14.5	7.3
30-40 weeks	1.0	0.5	1.5	3.0	30.0	15.0	7.5
Lactating women	3.45	0.5	1.5	5.45	54.5	27.3	13.7

Table 3 Recommended dietary allowances (RDA) for Zn [27].

Age, years		Infant	Children	Males	Females	Pregnant	Lactating	
							1st	2nd
	0-0.5	0.5-1.0	1-10	11-51 +	11-51 +		6mo	6mo
Zn RDA (mg)	5	5	10	15	12	15	19	16

4. Zn and Biological Functions

4.1 Enzyme Activities

Zn is essential for the activity of at least 90 enzymes which participate in all the major metabolic pathways. Over 40 metalloenzymes exist in which Zn is bound tightly to the enzyme in specific stoichiometric ratios and in which it serves one or more structures, regulatory or catalytic functions [28]. Mammalian metalloenzymes include carbonic anhydrase, carboxypeptidases, aminopeptidases, alkaline phosphatase, alcohol, retinol, malate, lactate, glutamate and glyceraldehyde-3-phosphate dehydrogenases. Some metallo enzymes require additional metals for activity, cytosolic superoxide dismutase, for example, has a requirement for copper as well as for Zn. Other metalloenzymes have only been identified in lower species and the particular metal dependence of an enzyme varies between species. Both DNA and RNA polymerases are Zn metalloenzymes in *Escherichia coli*; it has not been established that these are metalloenzymes in mammals but studies on animals indicate that thymidine kinase activity and nucleic acid synthesis are Zn-dependent in mammals. In some oncogenic viruses the reverse transcriptase (that is, RNA-dependent DNA polymerase) is a Zn metalloenzyme [29]. There is evidence that Zn is important for optimal activity of aspartate transcarbamylase, aminolaevulinic acid dehydratase activities [28], ornithine transcarbamylase and in fatty acid metabolism [30, 31]. Zn-deficient animals have impaired collagen synthesis and poor wound healing. This may reflect a generalized defect in protein and nucleic acid synthesis; however some evidence suggests that Zn deficiency alters the quantity and type of collagen cross links [32].

4.2 Cell Function

Zn is essential in all phases of the cell cycle, but, as yet, there is little evidence to relate this directly to its effects on nucleic acid and protein synthesis. Deficiency is associated with RNA desegregation and increased ribonuclease activity [33], and the ability of Zn to inhibit adenylate cyclase and phosphodiesterase may indicate a role in cell function and genetic expression by regulating the relative intracellular concentrations of cyclic-AMP and cyclic-GMP [34]. Cell replication rates in the oesophagus and pancreas of Zn -deficient rats are increased [35], suggesting that a loss of genetic regulation precedes an effect on nucleic acid and protein synthesis. In human lymphocytes Zn acts as a mitogen, but here the mechanism is unclear but appears to be mediated by monocytes [36]. Zn stabilises plasma and subcellular membranes [37] as well as nucleic acids and microtubules [38]; it stabilises lysosomes and high concentrations in vitro inhibit leucocytemobility and phagocytosis while enhanced macrophage migration has been described in Zn-deficient guinea-pigs [37]; membrane lipid peroxidation is increased in Zn deficiency states, and it has been proposed that Zn protects membranes from free radical oxidation.

4.3 Carbohydrate, Lipid and Protein Metabolism

Due to effect on hormone receptors, transcription factors and enzyme systems, Zn-containing enzymes and proteins participate in the metabolism of nucleic acids, proteins, carbohydrates and lipids. Zn has been shown to stimulate muscle glycolysis and inhibit hepatocyte glycogen synthesis [39]. MT/T also has an effect on Zn control of carbohydrate metabolism [40].

4.4 Effect of Zn Supplementation on Vitamin Status

Population have higher rates of nutritional deficiency, in particular marginal Zn deficiency can contribute to various chronic and degenerative diseases associated with aging. Vitamin A levels are significantly increased proportionally with Zn dose (for 30 mg Zn/day $P < 0.05$; for 15 mg Zn/day $P < 0.0001$), and the effect of Zn is more important after six months than three months of supplementation; no effect on vitamin E/cholesterol and erythrocyte folates [41].

4.5 Zn and Status of Other Micronutrients

The balance among micronutrients within the body appears to be finely regulated and it is therefore very important, particularly in the elderly, for whom deficiency in one or several micronutrients may have functional consequences on health. High intakes of Zn depress copper (Cu) absorption and decrease Cu status by stimulating the formation of metallothionein (MT). A high level of MT prevents Cu absorption/uptake in the intestine, liver, and kidney. Cu deficiency adversely affects lipid metabolism by decreasing HDL-cholesterol [42] and predisposes to cardiovascular abnormalities, by a mechanism which may involve free radical scavenging [43]. Moreover, Cu deficiency impairs lysyl oxidase function, an indispensable enzyme for structural integrity of vascular connective tissues [44]. Recent studies show that Zn does not reduce Fe absorption, by virtue of the fact that the DMT1 is not implicated in the intestinal Zn absorption [45]. In further support of this argument, a family of human intestinal Zn transporters (ZIP) was recently identified, suggesting separate mechanisms for Fe and Zn absorption [46]. In the case of vitamins, marginal, or low Zn status has been shown to decrease absorption of food folate, because the brushborder membrane folate conjugase, responsible for cleaving folate prior to absorption, is a Zn-dependent enzyme. Zn is necessary for the synthesis of hepatic cellular retinol-binding protein, which is essential for the intracellular transport of vitamin A in addition to its well established extracellular transport role [47]. Consequently, marginal Zn intake is associated with decreased mobilization of retinol from the liver and also with a lowered concentration of transport proteins in the blood, such as albumin, pre albumin, and transferring.

4.6 Immunological Effects

Zn plays a key role in multisided cellular and molecular mechanisms [48, 49]. For instance, Zn influences the lymphocyte response to mitogens and cytokines, serves as a co-factor for the thymic hormone thymulin, and is involved in leukocyte signal transduction [50-52]. An influence of Zn excess on T cell function was observed in several *in vitro* studies. In cell culture, very high Zn concentrations (above 100 μM) in a serum-free culture medium stimulate monocytes to secrete pro-inflammatory cytokines but actually inhibit T cell functions [53]. In general, T cells have a lower intracellular Zn concentration and are more susceptible to increasing Zn levels than monocytes [54, 55]. Also, *in vitro* allo reactivity was inhibited in the mixed lymphocyte reaction (MLC) after treatment with more than 50 μM Zn [56]. A similar inhibition was observed when the MLC was done *ex vivo* with cells from individuals that had been supplemented with 80 mg Zn per day for one week, indicating that Zn supplementation has the potential to suppress the allogeneic immune response at relatively low doses [57]. An *in vivo* study supported the finding that Zn excess can affect the lymphocyte function. Eighty three healthy volunteers ingested 330 mg Zn/d in three doses for a month. The treatment had a small but significant influence on the lymphocyte response to the mitogens phytohemagglutinin (PHA) and concanavalin A (Con A). Interestingly, it was observed that Zn had an immuno-regulatory influence, i.e., it decreased the lymphocyte response in high responders and had an enhancing effect on low responders [58].

4.7 Endocrine Function

Insulin is stored in the five-cells of the pancreas as a hexamer with two atoms of Zn [59], and Zn appears to

influence insulin binding and degradation at the hepatocyte plasma membrane [60]. In some studies impaired glucose tolerance and insulin response have been associated with Zn deficiency, but this has not been a consistent feature [61]. Prostatic androgen metabolism is modified by the intracellular concentration of Zn, and both high and low tissue concentrations inhibit the transformation of testosterone to dihydrotestosterone (DHT) [62, 63] demonstrated improved sperm counts and testosterone levels in hypozincaemic oligospermic males as a result of Zn administration; this treatment has also reversed uraemic impotence and increased DHT levels in patients with renal dialysis [64].

4.8 Hematology and Coagulation

Zn is an important constituent of RBCs. More than 90% of erythrocyte Zn is associated with carbonic anhydrase and Cu/Zn-SOD [65]. Zn deficiency results in decreased erythocyte precursors in the bone marrow and increased osmotic fragility of RBC membranes, possibly due to an abnormal sulfhydryl redox state and impairment of calcium uptake affecting potassium channels [66, 67]. In humans Zn deficiency is associated with impaired platelet aggregation and increased bleeding time [66, 68] is required for effective platelet calcium uptake from the extracellular space, likely through maintenance of calcium channels [66]. Calcium is required for enzyme activation to initiate the aggregation process [68]. Platelets accumulate Zn within a-granules and the cytoplasm, releasing it into the local environment in the clotting process. Through its interactions with plasma clotting factors, Zn plays a role in regulation of the balance of prothrombotic and antithrombotic factors [68].

4.9 Redox Reactions and Antioxidant

Zn directly protects the lipids and proteins of cellular membranes and thiol-dependent macromolecules (enzymes, tubulin and microtubules) from oxidative damage [66, 69, 70]. Zn depletion results in an abnormal thiol redox state in cellular membranes producing increased osmotic fragility of RBC membranes and inactive membrane calcium channel proteins [66]. Vitamin E, which scavenges intermediate peroxyl radicals, also protects membranes from lipid peroxidation; however, unlike Zn, it is not protected against the oxidation of protein sulfhydryl groups. With Zn deficiency, vitamin E levels decline, likely due to decreased intestinal lymphatic absorption as well as increased utilization as an antioxidant substitute for Zn. NADPH oxidases, enzymes that are associated with the plasma membrane and catalyze the production of the superoxide radical, are inhibited by Zn [71]. Zn is also an integral component of the antioxidant enzymes Cu/Zn-SOD, catalase and peroxidase [72]. Indirectly, Zn appears to act by inhibiting redox sensitive transcription factors and the primary intracellular antioxidant, GSH. Although the cause and effect relationships are not clear, cellular depletion of both Zn and GSH appear to be interrelated [69, 73].

As discussed above, Zn also induces MT/T production and helps maintain T in the reduced state [74]. Genetic damage can occur due to oxidative effects on DNA. Zn is a component of the structure and function of the transcription protein p53 and other DNA repair proteins [75]. Zn thus indirectly reduces potential free radical formation and lipid peroxidation, and protein and DNA oxidative injury.

4.10 Protection against Metal Toxicity and Other Noxious Agents

Zn, as well as MT/T, protects the cell against the toxic effects of metal ions such as iron, copper, mercury and cadmium [76, 77]. Zn also protects against carbon tetrachloride hepatotoxicity and ultraviolet or gamma irradiation, likely through Zn-dependent metalloenzyme regulation or antioxidant membrane-stabilizing effects [78, 79].

4.11 Reproduction

Zn is required in humans for spermatozoa

development, ovulation, fertilization, normal pregnancy, fetal development and parturition. Male hypogonadism seen with Zn deficiency appears to be associated with alterations in testicular steroidogenesis or Leydig cell failure [80, 81]. In humans, serum testosterone concentrations are directly correlated with cellular Zn concentrations. Zn is required for leydig cells to produce testosterone and for the enzymatic conversion to the biologically active 5a-dihydrotestosterone [82]. Zn decreases the hepatic clearance of testosterone and can increase its action, as the intracellular androgen receptor is a Zn finger protein [82, 83]. Zn also appears to be required for normal spermatogenesis and sperm function as it stabilizes chromatin, head-tail attachment, penetration, and fertilization [65, 82]. The concentration of Zn in the testes and prostate is very high, with the prostate secreting high levels in the semen [82]. In humans, seminal Zn concentration has been positively correlated with sperm motility, viability, and density. Zn's role in human female reproductive issues is not as clearly defined due to compound micronutrient deficiencies in the women being studied [84]. Studies suggest that Zn deficiency may increase the risk of fetal abnormalities, abortions, inefficient uterine contractions, and preterm labor and delivery [79, 85].

4.12 Zn and Bone Metabolism

Osteoporosis is a common age-related condition, which is a major cause of morbidity and mortality in the elderly of both genders. Subclinical Zn deficiency, due to a reduced dietary intake and/or impaired intestinal absorption of Zn may be a contributory factor for age-related osteoporosis [86]. For example, Zn is an essential cofactor for enzymes involved in the synthesis of various bone matrix constituents [87], and it plays a particularly important role in the regulation of bone deposition and resorption. Zn also plays a structural role in the bone matrix. Bone mineral is composed of hydroxyapatite crystals, which contain Zn complexes with fluoride. Zn is required for osteoblastic activity, directly by activating aminoacyl-tRNA synthetase in osteoblastic cells and stimulating cellular protein synthesis. Zn also promotes bone mineralization through its role as a cofactor of alkaline phosphatase [88]. *In vitro* studies have emphasized that Zn plays a role in the inhibition of bone resorption by inhibiting osteoclast-like cell formation [89]. In animals, Zn deficiency has been associated with abnormalities in bone growth, bone formation and mineralization [90]. Zn intake has been reported to be associated with low bone mass in women [91]. Furthermore, reduced serum or plasma Zn concentrations have also been reported in osteoporotic women [92, 93].

5. Physiological Function

5.1 Physical Growth

The impact of Zn on growth was first described in humans in adolescent populations in Iran and Egypt [94, 95]. In these studies of young adults who presented with a syndrome characterized by varying degrees of growth stunting and delayed sexual maturation (hypogonadal dwarfism), treatment with Zn induced accelerated growth and commencement of sexual maturation in most participants [95]. However, this response was not duplicated in a population of stunted and sexually delayed adolescents in a different area of Egypt, possibly because of other limiting nutrients in their diets [96].

In Fig. 3, prevalence of nutritional stunting in children less than five years of age children's growth may be due to its direct impact on nucleic acid and protein synthesis [97] and hormonal mediators of growth [98] or its effects on appetite [99, 100] or risk of infection, as discussed above. On the basis of this extensive research experience, there seems to be strong evidence of a relationship between Zn deficiency and growth stunting.

In those settings with high rates of stunting, underweight, low plasma Zn concentrations, or a combination of these factors and programs to enhance

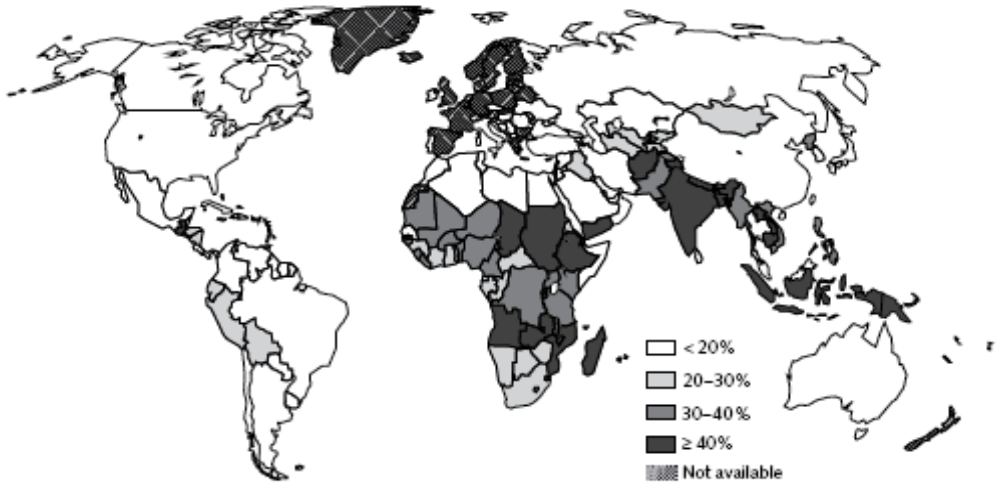


Fig. 3 Prevalence of nutritional stunting in children less than five years of age.
Source: the International Zn Nutrition Consultative Group (IZiNCG 2007).

the Zn status may be useful interventions to increase children's growth and decrease current rates of nutritional stunting.

WHO recommends a prevalence of stunting greater than 20% of the population to indicate a public health concern [27]. The highest prevalence rates of stunting > 30% are observed in countries in sub-Saharan Africa, South Asia and Southeast Asia. Intermediate prevalence rates 20% to 30% are found in the Andean countries, some Central American countries, Southern Africa, and some countries in North Asia. As Zn deficiency is not the only factor affecting children's growth, assessment of dietary Zn intake and serum Zn levels can be used to confirm the risk of Zn deficiency in these countries [101]. These assessments should be incorporated into existing public health and child nutrition monitoring programs whenever possible.

5.2 Hair Zn

Hair growth and the concentration of Zn in hair may be abnormal with Zn-responsive conditions in children however this test lacks sensitivity as the range of Zn levels overlaps with that of normal subjects [102, 103]. Normal hair Zn levels in Zn-deficient patients may be due to the rapid development of the deficient state (not yet identified in the hair samples), or a slowed hair growth rate from severe deficiency [103]. Suboptimal Zn diets capable of affecting hair follicle condition may not affect Zn levels in hair samples [104, 105].

5.3 Diarrhea

Zn deficiency increases the incidence of diarrhea and its supplementation reduces the prevalence. The role of Zn in diarrhea may be mediated through several mechanisms, which include membrane stabilization, mucosal integrity, electrolyte transport, water transport, immune-competence, protein and essential enzyme synthesis. The maintenance of the integrity of mucosal cell membranes, repair of mucosal injury by increased protein synthesis, multiplication of epithelial cells, and improvement of sodium and water transport is likely to reduce fluid loss during diarrhea. Repair of mucosal Para cellular tight junctions would allow better absorption of water. The improvement in immunological function especially on secretary IgA and T-lymphocytes would be expected to limit the growth and multiplication of diarrheal pathogens within the intestinal lumen [106].

5.4 Relation with Insulin and Diabetes Mellitus

Crystalline insulin contains a considerable amount of Zn (about 0.5%) and amorphous insulin also did not crystallize without the presence of Zn. Later it was

found that pancreatic islets contain Zn and many studies elucidate the exact role of Zn in the function and metabolism of the hormone and its relationship with diabetes mellitus. The role of Zn in insulin biosynthesis lies in the fact that pro insulin monomer binds the metal during its polymerization. It has been demonstrated that molecular organization in Zn proinsulin hexamer is maintained through all the subsequent steps in insulin biosynthesis. Zn is believed to be involved in insulin biosynthesis, hexamer formation and retention of its activity under storage condition and during mobilization. So if Zn deficiency occurs, it will reduce insulin's effect gradually paltering from the initial high level and finally diminishing to the base position. The reduced level of blood insulin activity, aggravated by high blood sugar and fat concentration, onsets adult or maturity diabetes (type II) in obese subjects having a history of hereditary predisposition and metabolic dysfunctions, occasionally leading to functional failure of the islet cells and pancreatic cyst formation [106].

5.5 Ageing

During ageing, the intake of Zn decreases, thus contributing to cause frailty, general disability and increased incidence of age related degenerative diseases (cancer, infections and atherosclerosis). Special focus on the role played by Zn in counteracting stress in ageing though the activity of some Zn dependent enzymes and in modulating apoptosis [107]. In this contest, Zn has been found a relevant modulator of the apoptosis especially in preventing the cell death at physiological dose whereas higher doses of Zn seem to activate apoptosis especially in presence of strong inducer of stress, such as dRib, in peripheral blood mononuclear cells from old people. These aspects associated with the well known neurotoxic action of Zn in ischemia and other brain disturbance counteract the positive effect generally observed by a supplementation with physiological doses of Zn. However, these aspects might find an explanation in view of the fact that Zn may be considered an "hormetic factor", up regulating the stress response induced gene expression thus stimulating the related pathways of maintenance and repair. In other words, Zn might act by one side, as a possible factor in eliminating damaged cells that may become potentially harmful and, by the other side, might help to select immune cells resistant to stress. This role of Zn may be exerted in many systems, tissues and organs, including the brain with a role in strengthening the brain functions and cognitive performances. In this context, a relevant role is played by metallothionein polymorphism in which old subjects with determinate alleles might have a major Zn ion bioavailability through a better Zn release by MT itself. Therefore, Zn may be useful in order to achieve health longevity escaping some age related diseases, such as atherosclerosis, perhaps thought an "hormetic effect".

5.6 The Integument and Wound Healing

The high epithelial proliferation rate of the skin places a high demand on Zn supply [108]. Zn is an essential cofactor for the conversion of linoleic acid to arachidonic acid in fatty acid metabolism [109]. The probable synergism between Zn and linoleic acid plays a role in keratinocyte proliferation and a reduction in transepidermal water loss [109-111]. EFA deficiency has been shown to impair Zn absorption; EFA supplementation enhances absorption [108]. The histologic abnormalities of necrolytic migratory erythema associated with pancreatic glucagonoma in humans are theorized to be a result of Zn, fatty acid, and amino acid deficiencies associated with malnutrition, malabsorption, liver disease, elevated glucagon levels, and metabolic derangements. Cutaneous wound healing consists of three stages: inflammation, proliferation, and remodeling. Zn deficiency or excess alters inflammatory responses causing delays wound closure [112].

5.7 Zn and Psychological Functions

About 90% of the total brain Zn is tightly bound to metalloproteins. Zn in the adult brain is located in the cerebral cortex [113], the "thinking" part of the brain. This region includes the hippocampus, which is assumed to play a role in episodic memory and spatial ability, and the amygdala or "feeling" part of the brain [114]. Zn is found in the presynaptic vesicles of glutamatergic neurons, which use glutamate as a transmitter. The role of Zn in these neurons is controversial but may include participation in the storage, release and uptake of glutamate, and modulation of glutamate receptors [115]. Zn can act as a neuromodulator or neurotransmitter [116]. As Zn deprivation may influence brain Zn homeostasis, it is an important nutrient for the brain function [117]. Evidence from the available literature suggests that both deficiency and excess of Zn may have profound positive and negative consequences, respectively, on human behaviour. Serum Zn concentrations have been associated with impaired cognitive function in older individuals [118]. Research has shown that certain micronutrients, including Zn, are significantly depleted in depressed patients [119] and Zn depletion has also been implicated in mood disorders [120, 121]. Furthermore, other studies of human subjects reported that Zn deficient individuals have declined taste acuity which can be restored by Zn supplementation [122]; however, the literature on this topic appears contradictory [123]. Zn deficiency has also been identified as a possible contributor to loss of appetite [124] and anorexia [125] by inhibiting the release of neuropeptide Y (NPY), which is required for receptor activation [126]. Indeed, NPY regulates a wide variety of physiologic functions [127] and it is also known to act as an orexigen (a stimulator of food intake).

5.8 Zn Toxicity

Zn has a low toxicity, although acute symptoms of nausea, vomiting, diarrhea, fever and lethargy may be observed when large (about 1 g) doses are consumed. When Zn intake exceeds physiological needs by reasonably small amounts, homeostasis can be maintained by increased endogenous fecal and urinary excretion. However, if excessive Zn intake continues for prolonged periods of time, absorption of other trace elements, especially copper and iron, can be impaired. For example, intakes of supplements providing 50 mg of Zn per day for six weeks produced changes in erythrocyte copper-Zn superoxide dismutase, an indicator of copper status [128, 129]. At higher doses of Zn (160-660 mg/day), anemia and changes in immune function and lipoprotein metabolism have been observed in addition to abnormal indices of copper status [129, 130]. Further studies are needed to specify the level of Zn intake at which any undesirable effects on copper metabolism, hematologic indices, immune function, and lipoprotein metabolism begin to occur. Moreover, it needs to be determined whether the recommended upper limit of Zn intake should be modified when it is consumed with other foods of varied phytate content. There is also some evidence that adverse effects of Zn are more likely to occur when copper intakes are low, and a Zn:copper molar ratio of no more than 15 has been suggested [131, 132].

6. Conclusions

Due to the multiple biologic functions of Zn and its ubiquitous distribution in human tissues, there is a broad range of physiological signs of Zn deficiency, which may vary depending on the affected individuals. The adverse consequences of Zn deficiency include impaired immune competence, impaired growth and development and impaired maternal health and pregnancy outcomes. These complications of Zn deficiency may be better defined when the specific biochemical mechanisms that link Zn status to these outcomes are elucidated. Although other factors may contribute to the development of Zn deficiency, inadequate dietary intake of absorbable Zn is likely to be the most common cause. The adequacy of Zn intake is affected by the presence of dietary factors that inhibit

Zn absorption, primarily the phytate:Zn molar ratio. Diets based largely on unprocessed cereals or tubers and negligible amounts of animal source foods increase the dietary requirements for Zn, and therefore heighten the challenge of acquiring an adequate amount of Zn from the diet. It is also recognized that Zn deficiency in many populations may be attributable to underlying social and economic problems, such as poverty, poor quality food supply and lack of nutrition education. Based on the large body of evidence for positive effects of supplemental Zn on multiple outcomes of concern to public health, it is evident that similar benefits would be realized in designed to improve Zn intakes. Identification of nutritional Zn deficiency and its specific causes has therefore become a growing concern for public health planners. So afterwards, more controlled and clinical studies to ascertain the effects and mechanisms of Zn on human appear necessary in view of the necessity to minimize infections.

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A Comparative Study of Stability of Extra Virgin Olive Oil, Virgin Coconut Oil and Grape Seed Oil against Domestic Deep Frying

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Abstract: The aim of this study was to evaluate the relative stability of extra virgin olive oil (EVOO), virgin coconut oil (VCO) and grape seed oil (GSO) against domestic deep frying. Oil samples were subjected to deep frying at 190 °C for 30, 60, and 90 min and then compared with fresh oil samples in terms of fatty acid composition, peroxide value (PV), p-anisidine value (p-AV), total oxidation value (TOTOX), iodine value (IV), free fatty acid content (%FFA) and total phenolic content (TPC). Experimental results showed that the changes in the fatty acid composition, p-AV and TOTOX were in the order, GSO > EVOO > VCO throughout the experiment, while PV was in the order, VCO > EVOO > GSO. Meanwhile, the reduction in the IV was in the order, GSO > VCO > EVOO throughout the experiment. On the other hand, the changes in the %FFA were in the order, VCO > GSO > EVOO throughout the experiment. VCO had the greatest stability against domestic deep frying, followed by EVOO and GSO had the least stability against domestics deep frying.

Key words: EVOO, VCO, GSO, oxidative stability, domestic deep frying.

1. Introduction

Vegetable oils become dominant in market partly due to increasing of public awareness about the negative health effects of the consumption of saturated fat and cholesterol. Unlike animal fats, vegetable oils contain no cholesterol, generally low in saturated fat, and are a good source of Vitamin E. With such composition, the consumption of vegetable oils has beneficial effects on human health, such as reducing the risk of cardiovascular disease and lowering blood low density lipoprotein (LDL) cholesterol level [1].

However, an important issue regarding the stability of vegetable oils under high temperature is raised when they are served as frying oils in the domestic deep frying process. Under these conditions, vegetable oils are difficult to be stabilized because of their high content of unsaturated fatty acids [2]. Unsaturated fatty acids are vulnerable to oxidation because this type of fatty acids contains at least one double bond where chemical reactions can be easily taken place [3]. As a result, various chemical reactions such as oxidation and hydrolysis of the components in vegetable oils will be performed rapidly during domestic deep frying [4].

Oxidation and degradation of different components in vegetable oils can result a variety of substances to be formed, including volatile carbonyls, hydroxyl acids, keto acids and epoxy acids [5]. Some of these degraded products have harmful effects on human health as they can destroy vitamins, inhibit enzymes and potentially cause mutations or gastrointestinal irritations [6]. Apart from that, some of the degraded products can also cause unpleasant odour and flavour in the deteriorated vegetable oils [7]. Although frying oils are not the main products in the domestic deep frying process, the quality and composition of frying oils will definitely affect the

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fried foods. It is because frying oils will migrate into foods during domestic deep frying and their content in fried foods can be ranged from 5% to 40% [8]. Hence, the stability of vegetable oils upon domestic deep frying is a key concern when it comes into the criteria of selecting of vegetable oils for domestic deep frying.

To date, many studies have been carried out to evaluate the stability of various vegetable oils (such as olive oil, sunflower oil, soybean oil and corn oil) against deep frying [9]. However, there is no much studies which have been done on the stability of virgin coconut oil (VCO), extra virgin olive oil (EVOO) and grape seed oil (GSO) against deep frying and thermal treatment. Besides that, the comparative study of the stability of these vegetable oils against domestic deep frying also has not been done in any study.

2. Materials and Methods

2.1 Materials

EVOO, VCO and GSO were purchased from local supermarket, Cheras, Malaysia.

2.2 Methods

2.2.1 Deep Frying Experiment

All deep frying experiments were performed using the same electrical deep fryer equipped with thermostat (Philips, Cucina DH 6151, China). Appropriate amount of French fries were removed from freezer and defrosted in chiller (3 °C) for 2 h before the deep frying experiment was carried out. For each deep frying experiment, 500 ± 1 mL of vegetable oil was preheated for about 15 min to reach 190 °C. Then, 30 ± 1 g of French fries were fried for 5 min and removed. Subsequently, the oil sample was heated for another 5 min without French fries. The procedures of frying French fries for 5 min and heating vegetable oil without French fries for 5 min were referred as one frying cycle. Every type of vegetable oils was subjected to deep frying for three different total frying cycles, which were three, six and nine total frying cycles, equivalent to the periods of 30, 60 and 90 min, respectively. Throughout the frying experiment, deep fryer lid was opened to induce oxidative deterioration of oil sample by allowing it to react with the atmospheric oxygen. All the deep frying experiments were done in replicate.

2.2.2 Analyses

Iodine value (IV), free fatty acid content (%FFA), peroxide value (PV) and p-anisidine value (p-AV) were determined according to AOAC [10]. While total oxidation value (TOTOX) was determined according to the following equation:

$$TOTOX = 2 \times PV + p\text{-}AV \quad (1)$$

2.2.3 Fatty Acid Composition

The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 μL of n-hexane to 50 mg of oil followed by 50 μL of 30 mL/100 mL sodium methoxide in methanol [11]. The mixtures were vortexed for 5 s and allowed to settle for 5 min. The top layer (1 μL) was injected into a gas chromatograph (Hewlett-Packard Model 5890 instrument (Palo Alto, CA, USA)), equipped with a flame-ionization detector (FID) and a Hewlett-Packard Model 3392A integrator. A polar capillary column BPX70 (0.32 mm internal diameter, 30 m length and 0.25 μm film thickness; SGE International Pty. Ltd., Victoria, Australia) was used at a column head pressure of 10 psi. Helium (99.995%) at approximately 23 mL/min (measured at oven temperature 150 °C) was used as the carrier gas, and nitrogen (99.999%) at 20 mL/min was used as the makeup gas. The FID and injector temperatures were both maintained at 220 °C. The initial column oven temperature was 115 °C, temperature programmed to 180 °C at 8 °C/min and held at this temperature until the analysis was completed. FAME peaks were identified by comparison of retention times to a Supelco 37 component FAME mix (obtained from Sigma-Aldrich, St. Louis, US). The peak areas were computed, and percentages of FAME were obtained as area percentages by direct normalization (the data are expressed as normalized percent of all identified

FAME). Only the more abundant FA (> 0.2%) was considered. All analyses were carried out in triplicate.

2.2.4 Total Phenolic Content (TPC)

Extraction of phenolic compounds from oil samples was done according to the method described by Rotondi et al. [12] with some modification. 5.0 ± 0.1 g of oil sample was weighted into 50 mL Falcon tube. 5 mL of hexane was then added to dissolve oil sample by means of vortex. The mixture was extracted with 10 mL of methanol:water (60:40, v/v). The mixture was vortexed for 5 min and subsequently centrifuged at 4,500 rpm for 5 min. The methanolic phase was collected and the hexane phase was re-extracted twice with 10 mL of methanol:water (60:40, v/v) each time. The combined methanolic fractions from three extractions were subjected to final washing with 10 mL of hexane to eliminate residual oil sample in a separating funnel. The methanolic fractions were evaporated under reduced pressure at 40 °C until dryness using rotary evaporator. The residue was then reconstituted with 8 mL of methanol:water (60:40, v/v) for EVOO sample and 2 mL of methanol:water (60:40, v/v) for GSO and VCO sample.

2.2.5 TPC Assay of Methanolic Extract

TPC of methanolic extract was determined in the same way as gallic acid standards by replacing the gallic acid standard with methanolic extract. The result was expressed as mg of gallic acid equivalent per 100 g of oil sample (mg GAE/100 g of oil) and calculated based on the equation below:

$$TPC = \frac{Ab \times V \times 100}{17.18 \times Wt} \quad (2)$$

where, Ab = absorbance of methanolic extract at 765 nm; wt = weight of oil sample (g) and V = volume of methanol:water (60:40, v/v) used to reconstitute the extract after evaporating until dryness (mL).

2.3 Statistical Analysis

All the deep frying experiments and preparation of fresh oil samples were done in replicate sets while analyses were performed in triplicate. The experimental results were recorded as mean ± standard deviation. The results were analysed using one-way analysis of variance (ANOVA) by MINITAB software (Version 14.1.1.0), in which frying period was the factor while the values of experimental results were chosen as responses. The significant differences ($P < 0.05$) among the results were determined along with the Tukey's test in this study.

3. Results and Discussion

3.1 Changes in IV of Oil Samples during Deep Frying Experiment

The changes in the IV of EVOO, VCO and GSO throughout the deep frying experiment are presented in Fig. 1. Oxidation and degradation of PUFA and MUFA would be taken place when oil samples were subjected to thermal treatment [9]. The loss of PUFA and MUFA in oil samples caused a reduction in the level of unsaturated double bonds.

Among these three different types of oil samples, GSO experienced the greatest loss of IV throughout the deep frying experiment, followed by VCO. EVOO experienced the least loss of IV during the deep frying experiment, in which its IV had no significant decrease ($P < 0.05$) in the first 60 min of deep frying experiment. EVOO only experienced a significant loss ($P < 0.05$) of IV after subjecting it to deep frying for 90 min, in which it lost a total IV of 4.1 g I_2/100 g of oil. These results also indicated that GSO experienced the greatest loss of unsaturated double bonds, followed by VCO, and EVOO experienced the least loss of unsaturated double bonds during the deep frying experiment.

GSO experienced the greatest loss in its IV during the deep frying experiments due to its consisting of higher percentage of PUFA but lower percentage of MUFA and SFA than EVOO and GSO. Oils with high IV are prone to oxidise and degrade more rapidly than those with low IV [13]. In other word, the loss of

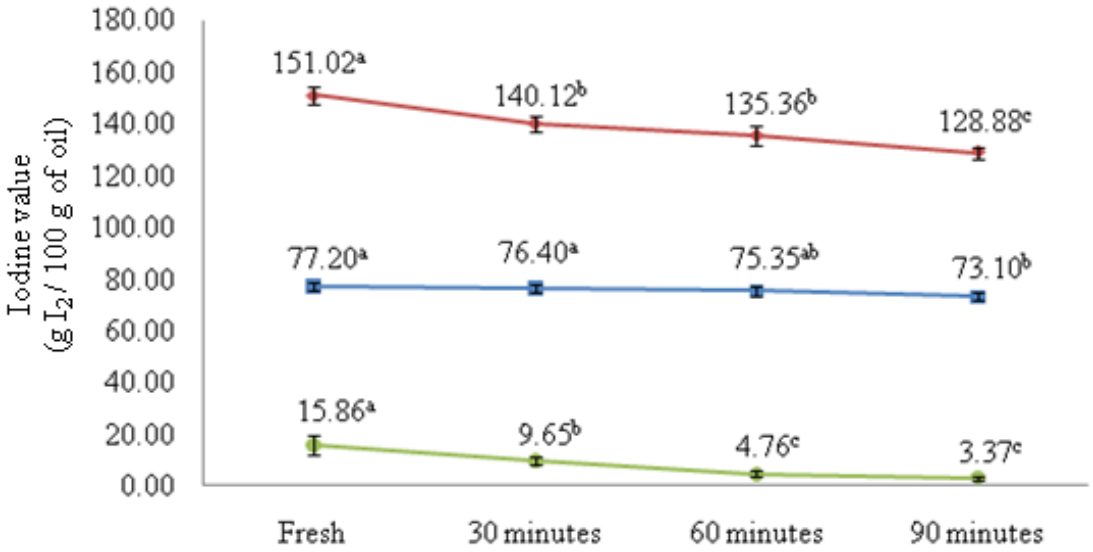

Fig. 1 IV of EVOO, VCO and GSO before and after deep frying for 30, 60 and 90 min.
(EVOO; GSO; VCO)
Each value from the figure represents the mean (n = 3), whereas the error bar represents the standard deviation. Means within each type of vegetable oils bearing with different superscripts show significantly different (P < 0.05).

unsaturated double bonds in oils with high IV would be performed more rapidly than those with low IV during the deep frying experiment. However, the changes in the IV of VCO and EVOO during the deep frying experiment showed contradiction to this concept. EVOO (which had higher IV than VCO) experienced lesser loss of IV than VCO throughout the deep frying experiment. This circumstance could be due to VCO had lower phenolic content and higher level of free fatty acid than EVOO, as evidenced by oil samples' TPCs that are shown in Table 1 and oil samples' %FFAs that are shown in Fig. 2. With low level of phenolic compounds in VCO, the antioxidant activity within VCO may not be effective to protect PUFA and MUFA from oxidation and degradation during the deep frying experiment. In addition, high content of free fatty acids in VCO could also accelerate the oxidation and degradation of PUFA and MUFA during deep frying experiment. This is due to the carbonyl group on free fatty acid had catalytic effect on the formation of free radicals by decomposition of hydroperoxides [14]. All these factors might work together to reduce the stability of VCO and resulted in a greater reduction in its degree of unsaturation as compared to EVOO during the deep experiment.

Additionally, a reduction in the rate of the loss of IV was found in VCO as the deep frying experiment was progressed to 90 min. This phenomenon could be due to the availability of unsaturated fatty acids in VCO become limited as the deep frying experiment was performed to 90 min. Based on the fatty acid composition of fresh VCO that is shown in Table 2, VCO contained limited unsaturated fatty acids, in which these fatty acids only accounted 5.63% of the total fatty acids content.

3.2 Changes in %FFA of Oil Samples during Deep Frying

The changes in the %FFA of EVOO, VCO and GSO throughout the deep frying experiment are showed in Fig. 2. Generally, GSO experienced gradual increase in its %FFA while EVOO experienced no significant change (P < 0.05) in its %FFA as the progression of deep frying experiment. The %FFA of VCO was observed to be substantial decrease in the first 30 min of the deep frying experiment and the decreasing of %FFA in VCO became gradual after 30 min of the deep frying experiment.

Table 1 TPC of EVOO, GSO and VCO before and after deep frying for 30, 60 and 90 min (n = 3)*.

Time of deep frying (min)	Total phenolic content (mg GAE/100 g of oil)**		
	EVOO	GSO	VCO
0 (Fresh)	15.84 ± 0.56^{a}	2.40 ± 0.04^{a}	0.66 ± 0.02^{a}
30	7.54 ± 0.32^{b}	1.14 ± 0.05^{b}	0.51 ± 0.03^{b}
60	5.44 ± 0.16^{c}	0.85 ± 0.14^{c}	0.49 ± 0.02^{b}
90	3.97 ± 0.14^{d}	0.60 ± 0.13^{d}	0.31 ± 0.03^{c}
Total loss (%)	74.94	75.00	53.03

*Replicate.

**Each value from the table represents the mean ± standard deviation. Means within each column with different superscripts are significantly different ($P < 0.05$).

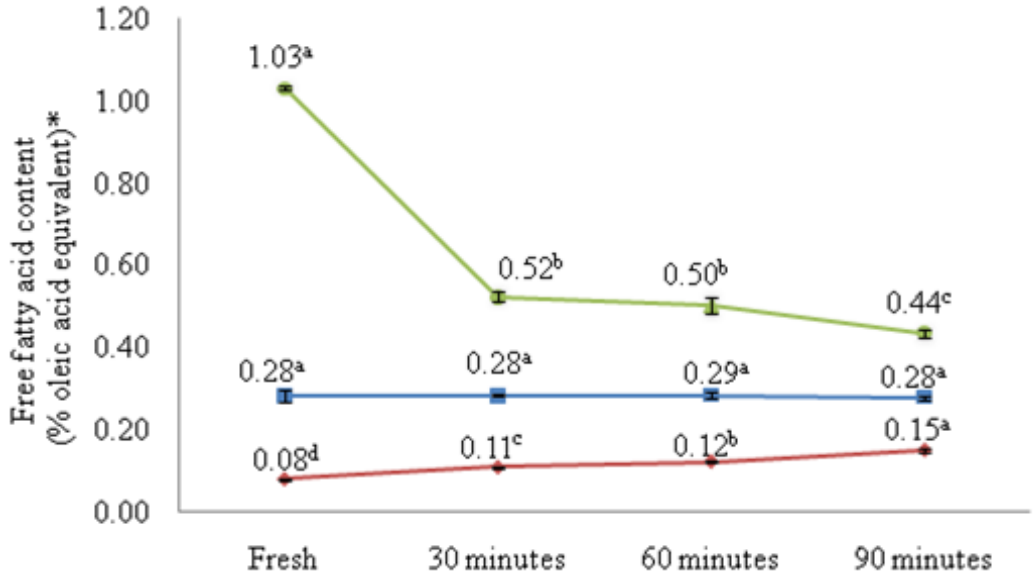

Fig. 2 %FFA of EVOO, VCO and GSO before and after deep frying for 30, 60 and 90 min.

(EVOO; GSO; VCO)

Each value from the figure represents the mean (n = 3), whereas the error bar represents the standard deviation. Means within each type of vegetable oils bearing with different superscripts show significantly different ($P < 0.05$).

The %FFA of EVOO was observed to be no significant change ($P < 0.05$) throughout the deep frying experiment. One possible reason that EVOO experienced no significant change ($P < 0.05$) in its %FFA throughout the deep frying experiment could be due to it had high antioxidant content, particularly phenolic compounds, as evidenced by its TPC that is shown Table 1. Therefore, the presence of high phenolic compounds in EVOO might reduce the formation rate of FFA from the hydrolysis of TG and/or decomposition of aldehydes into certain threshold, in which no significant increase ($P < 0.05$) in %FFA was observed throughout the 90 min deep frying experiment.

When vegetable oils are subjected to thermal treatment in the presence of moisture from foods, hydrolysis must be taken place [15]. Therefore, experiencing a reduction in %FFA in VCO throughout the deep frying experiment suggested that the degradation of FFA occurred at the same time of the formation of FFA from hydrolysis. Moreover, the degradation of FFA performed faster than the formation of FFA from hydrolysis. This circumstance could be due to VCO had an unusual high initial %FFA. Conversely, VCO had a %FFA of 1.03% oleic acid equivalent. High %FFA in VCO may accelerate the

Table 2 Fatty acid composition of (a) EVOO, (b) VCO, (c) GSO before and after deep frying for 30, 60 and 90 min (n = 3)*.

(a)

Fatty acid	Percentage of fatty acid (%)**			
	Fresh oil sample	Deep frying for 30 min	Deep frying for 60 min	Deep frying for 90 min
Palmitic acid ($C_{16:0}$)	14.09 ± 0.28^{b}	14.44 ± 0.13^{b}	14.90 ± 0.77^{ab}	15.76 ± 0.27^{a}
Palmitoleic acid ($C_{16:1}$)	1.26 ± 0.07^{b}	1.54 ± 0.02^{a}	1.61 ± 0.11^{a}	1.41 ± 0.19^{ab}
Stearic acid ($C_{18:0}$)	2.14 ± 0.10^{a}	2.32 ± 0.29^{a}	2.59 ± 0.20^{a}	2.33 ± 0.19^{a}
Oleic acid ($C_{18:1}$)	71.20 ± 4.38^{a}	72.02 ± 3.39^{a}	71.67 ± 3.74^{a}	71.82 ± 3.83^{a}
Linoleic acid ($C_{18:2}$)	11.31 ± 1.14^{a}	9.68 ± 0.78^{b}	9.24 ± 0.24^{b}	8.68 ± 0.15^{b}
SFA	16.23 ± 0.26^{c}	16.78 ± 0.41^{bc}	17.49 ± 0.73^{ab}	18.09 ± 0.21^{a}
MUFA	72.42 ± 1.19^{a}	73.50 ± 0.81^{a}	73.27 ± 0.49^{a}	73.23 ± 0.11^{a}
PUFA	11.35 ± 1.14^{a}	9.71 ± 0.78^{b}	9.24 ± 0.24^{b}	8.68 ± 0.15^{b}

(b)

Fatty acid	Percentage of fatty acid (%)**			
	Fresh oil sample	Deep frying for 30 min	Deep frying for 60 min	Deep frying for 90 min
Caprylic acid ($C_{8:0}$)	8.82 ± 0.05^{a}	8.46 ± 0.13^{b}	8.77 ± 0.11^{a}	8.65 ± 0.14^{a}
Capric acid ($C_{10:0}$)	6.97 ± 0.10^{a}	6.88 ± 0.06^{a}	6.90 ± 0.07^{a}	6.90 ± 0.03^{a}
Lauric acid ($C_{12:0}$)	50.27 ± 0.31^{a}	50.19 ± 0.41^{a}	50.31 ± 0.33^{a}	49.47 ±0.20^{a}
Myristic acid ($C_{14:0}$)	17.88 ± 0.03^{a}	17.99 ± 0.16^{a}	18.01 ± 0.08^{a}	17.77 ± 0.19^{a}
Palmitic acid ($C_{16:0}$)	7.82 ± 0.12^{b}	8.32 ± 0.07^{b}	8.40 ± 0.15^{b}	9.07 ± 0.54^{a}
Stearic acid ($C_{18:0}$)	2.61 ± 0.22^{a}	2.89 ± 0.21^{a}	2.80 ± 0.23^{a}	2.99 ± 0.15^{a}
Oleic acid ($C_{18:1}$)	4.40 ± 0.33^{a}	4.29 ± 0.18^{a}	4.00 ± 0.29^{a}	4.25 ± 0.29^{a}
Linoleic acid ($C_{18:2}$)	1.23 ± 0.07^{a}	0.98 ± 0.04^{b}	0.81 ± 0.08^{c}	0.60 ± 0.11^{d}
SFA	94.36 ± 0.38^{b}	94.73 ± 0.21^{ab}	95.19 ± 0.31^{a}	95.36 ± 0.22^{a}
MUFA	4.41 ± 0.33^{a}	4.29 ± 0.18^{a}	4.00 ± 0.29^{a}	4.03 ± 0.29^{a}
PUFA	1.23 ± 0.07^{a}	0.98 ± 0.04^{b}	0.81 ± 0.08^{c}	0.61 ± 0.11^{d}

(c)

Fatty acid	Percentage of fatty acid (%)**			
	Fresh oil sample	Deep frying for 30 min	Deep frying for 60 min	Deep frying for 90 min
Palmitic acid ($C_{16:0}$)	7.08 ± 0.20^{b}	7.64 ± 0.17^{b}	7.77 ± 0.28^{b}	9.34 ± 0.83^{a}
Stearic acid ($C_{18:0}$)	3.57 ± 0.65^{a}	4.11 ± 0.04^{a}	4.10 ± 0.05^{a}	4.24 ± 0.13^{a}
Oleic acid ($C_{18:1}$)	18.65 ± 0.87^{a}	19.62 ± 0.36^{a}	21.49 ± 1.48^{a}	21.09 ± 1.93^{a}
Linoleic acid ($C_{18:2}$)	70.70 ± 0.60^{a}	68.63 ± 0.56^{ab}	66.64 ± 3.25^{ab}	65.33 ± 2.77^{b}
SFA	10.65 ± 0.52^{c}	11.75 ± 0.19^{b}	11.86 ± 0.23^{b}	13.58 ± 0.71^{a}
MUFA	18.68 ± 0.87^{a}	19.62 ± 0.32^{a}	21.51 ± 1.58^{a}	21.08 ± 1.60^{a}
PUFA	70.67 ± 0.60^{a}	68.63 ± 0.51^{ab}	66.63 ± 1.25^{b}	65.34 ± 2.30^{b}

*Replicate;

**Each value from the table represents the mean ± standard deviation. Means within each row with different superscripts are significantly different ($P < 0.05$).

oxidation and degradation of different component within it, including FFA themselves (14). Although the formation of FFA from hydrolysis was taken place, the degradation of FFA by oxidation and other degradation reactions in VCO might be performed more rapidly. Hence, a substantial reduction in the %FFA of VCO was observed in the first 30 min of deep frying experiment. However, as the deep frying experiment was progressed to 90 min, the rate of the reduction of %FFA in VCO was observed to be generally reduced. This trend could be due to the catalytic effects of FFA on the oxidation and degradation of FFA was reduced as the level of FFA in VCO was reducing with periods of deep frying. As a result, the loss of %FFA in VCO became gradual as the progression of deep frying experiment.

3.3 Changes in PV and p-AV of Oil Samples during Deep Frying

The changes in the PV and p-AV of EVOO, GSO and VCO throughout the deep frying experiment are shown in Figs. 3 and 4, respectively. In general, both PVs and p-AVs of all oil samples were increased with periods of deep frying experiment.

VCO was observed to have the highest PV, followed by EVOO and GSO had the lowest PV throughout the deep frying experiment (Fig. 3). In terms of p-AV, all oil samples experienced substantial increases in p-AV after using for deep frying for 30 min (Fig. 4). In the subsequent 60 min of the deep frying experiment, the p-AVs of both EVOO and GSO were increased with periods of deep frying; whereas the p-AV of VCO was observed to be no significant change ($P < 0.05$), in which its p-AV was remained at a value around 5.50. Apart from that, VCO was observed to have the lowest p-AV, followed by EVOO and GSO had the highest p-AV among all oil samples throughout the deep frying experiment.

In most situations, vegetable oils with high PV are accompanied with high p-AV and this condition indicates that the vegetable oils are highly oxidised [16]. However, the PV and p-AV of VCO showed a contradiction to their findings when its was compared to the PVs and p-AVs of EVOO and GSO during the deep frying experiment. Throughout the deep frying experiment, VCO had higher PV than EVOO and GSO, but its p-AV was lower than EVOO and GSO. Similar situation was also observed in the case of EVOO and GSO. EVOO had higher PV than GSO but its p-AV was lower than GSO throughout the deep frying experiment.

Based on the fatty acid compositions of oil samples that are shown in Table 2, VCO had the lowest percentage of unsaturated fatty acids (5.64%), followed by EVOO (83.77%), and GSO had the highest percentage of unsaturated fatty acids (89.35%). Therefore, during the deep frying experiment, VCO might experience the lowest decomposition rate of hydroperoxides, followed by EVOO and GSO. When

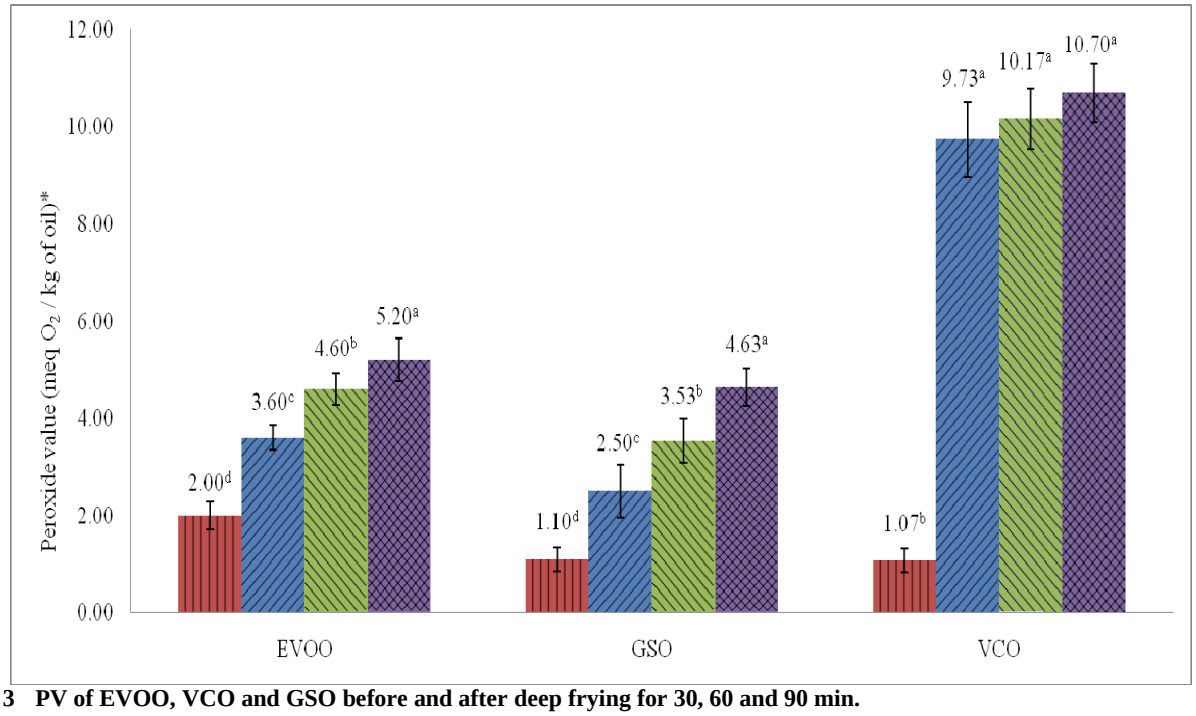

Fig. 3 PV of EVOO, VCO and GSO before and after deep frying for 30, 60 and 90 min.
(Fresh; 30 min; 60 min; 90 min)
Each value from the figure represents the mean ($n = 3$), whereas the error bar represents the standard deviation. Means within each type of vegetable oils bearing with different superscripts show significantly different ($P < 0.05$).

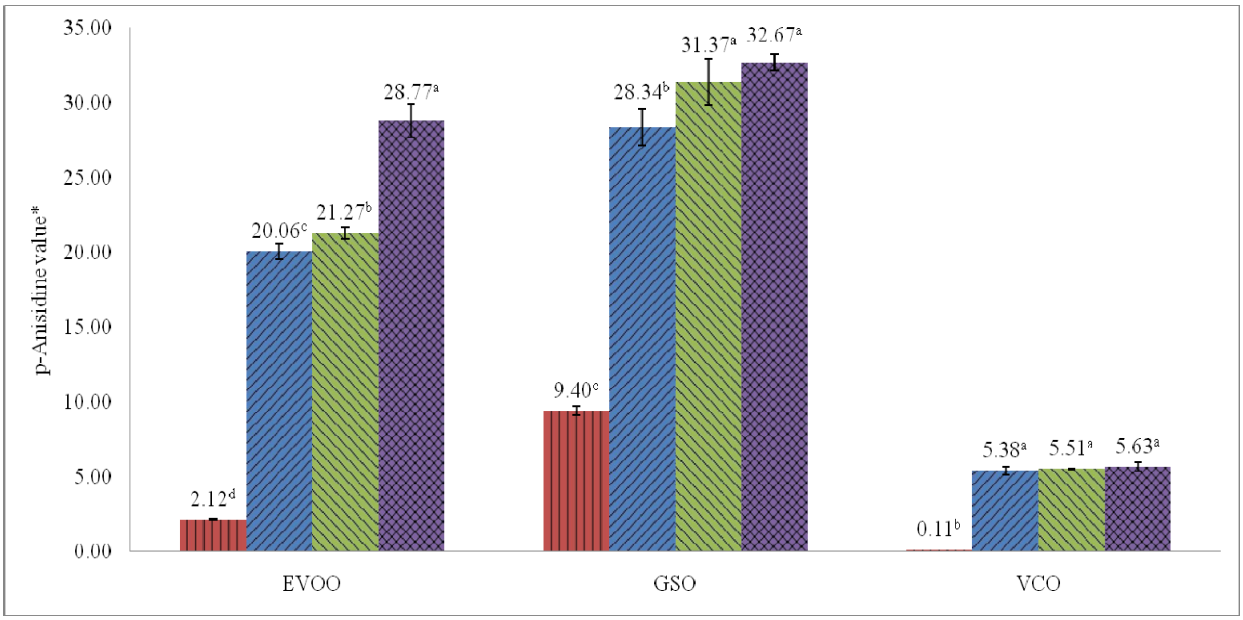

Fig. 4 p-AV of EVOO, VCO and GSO before and after deep frying for 30, 60 and 90 min.
(Fresh; 30 min; 60 min; 90 min)
Each value from the figure represents the mean (n = 3), whereas the error bar represents the standard deviation. Means within each type of vegetable oils bearing with different superscripts show significantly different ($P < 0.05$).

the decomposition of hydroperoxides was performed slowly in oil samples, high level of hydroperoxides would be retained while low level of aldehydes would be formed from the decomposition of hydroperoxides or vice versa. As a result, VCO had the highest level of hydroperoxides but the lowest level of aldehydes, followed by EVOO, and GSO had the lowest level of hydroperoxides but the highest level of aldehyes during the deep frying experiment.

3.4 Changes in TOTOX of Oil Samples during Deep Frying

The changes in the TOTOX of EVOO, GSO and VCO throughout the deep frying experiment are presented in Fig. 5. Generally, the TOTOXs of all oil samples were increased with periods of deep frying.

Among three different oil samples, VCO had the lowest TOTOX throughout the deep frying experiment, followed by EVOO and GSO. These results indicated that VCO experienced the least degree of oxidation, followed by EVOO and GSO experienced the greatest degree of oxidation during the deep frying experiment.

The phenomena of having different TOTOX in different oil samples during the deep frying experiment could be explained based on oil samples' degree of unsaturation. GSO had the highest degree of unsaturation, followed by EVOO and VCO had the lowest degree of unsaturation (Fig. 1). Therefore, during the deep frying experiment, the oxidation reaction in GSO would be performed most rapidly, followed by EVOO and the oxidation reaction in VCO would be performed in the lowest rate.

3.5 Changes in the TPC of Oil Samples during Deep Frying Experiment

The TPCs of EVOO, GSO and VCO at different intervals of the deep frying experiment are recorded in Table 1. Basically, the TPCs of all oil samples were decreased with periods of deep frying. This circumstance was possible due to the phenolic compounds in oil samples were destroyed by thermal destruction and/or contributing antioxidant activity to the oil samples for purpose of preventing and/or slowing down the oxidative reactions that were carried out in oil samples.

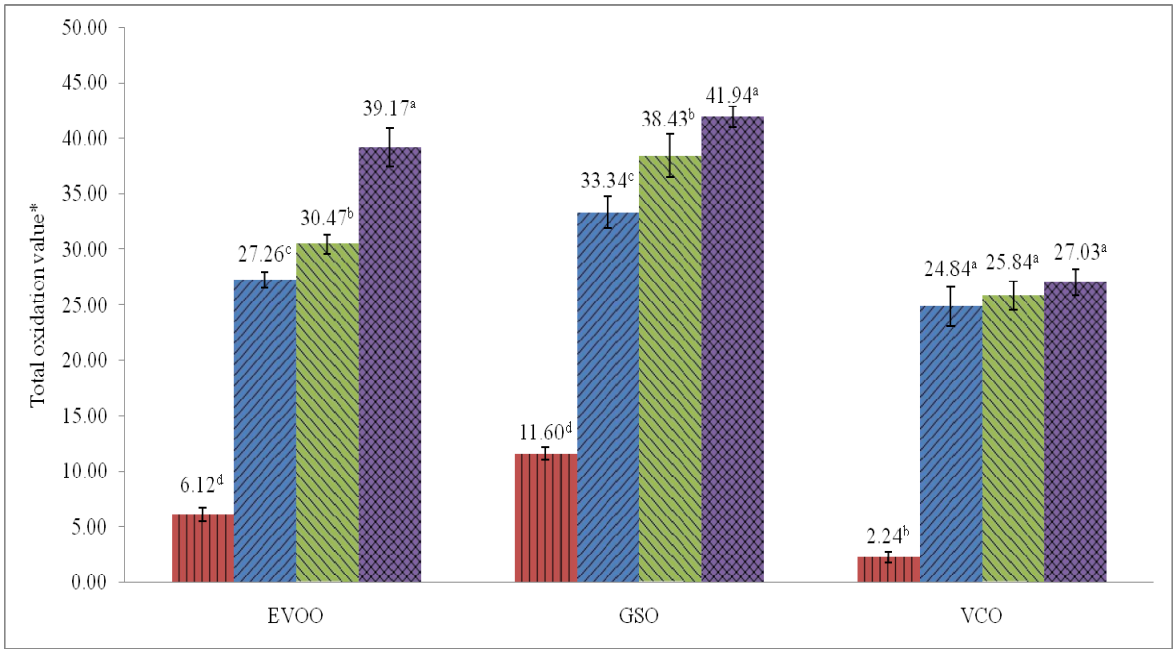

Fig. 5 Total oxidation values of EVOO, VCO and GSO before and after deep frying for 30, 60 and 90 min.
(Fresh; 30 min; 60 min; 90 min)
Each value from the figure represents the mean (n = 3), whereas the error bar represents the standard deviation. Means within each type of vegetable oils bearing with different superscripts show significantly different ($P < 0.05$).

The percentage losses of TPC in EVOO, GSO and VCO at different intervals of deep frying experiment were calculated in this study in order to determine the relative losses of TPC among three different oil samples during the deep frying experiment. As observed, the percentage losses of TPC in EVOO and GSO were almost the same and greater than VCO throughout the deep frying experiment. Among all oil samples, VCO experienced the least percentage loss of TPC at the end of deep frying experiment.

Experiencing a great percentage loss in the TPC of EVOO could be due to phenolic compounds which were the major antioxidants that contributed to the oxidative stability of EVOO during deep frying experiment. Phenolic compounds and tocopherols are the main antioxidants that are presented in virgin olive oil [17]. However, Baldioli et al. [18] revealed that the level of phenolic compounds in virgin olive oil was correlated (r = 0.97) with its oxidative stability but the level of tocopherol showed a low correlation (r = 0.05) with its oxidative stability. In other words, phenolic compounds might be the main natural antioxidants that contributed to the stability of virgin olive oil. Therefore, during the deep frying experiment, phenolic compounds might be decomposed rapidly by contributing antioxidant activity to EVOO for the purpose of preventing and delaying the oxidative deterioration of EVOO. As a result, the percentage loss of TPC in EVOO was very high during the deep frying experiment.

On the other hand, experiencing different percentage losses of TPC in GSO and VCO during the deep frying experiment could be due to GSO and VCO experienced different degree of oxidation during the deep frying experiment. When the oxidation reactions were performed rapidly in oil samples, the rate of the formation of free radicals in oil samples would also be increased or vice versa [19]. Therefore, the formation of free radicals in GSO would be performed most rapidly while the formation of free radicals in VCO would be performed most slowly during the deep frying experiment. Since phenolic compounds can act as primary antioxidants by scavenging the free radicals, experiencing high formation rate of free radicals in oil

samples would destroyed the phenolic compounds in oil samples rapidly or vice versa. As a result, GSO experienced a great percentage loss in TPC while VCO experienced less percentage loss in TPC during the deep frying experiment.

3.6 Changes in the Fatty Acid Composition of Oil Samples during Deep Frying Experiment

The changes in the fatty acid composition of EVOO, VCO and GSO during deep frying experiment are shown in Table 2. Overall, the fatty acid compositions of all oil samples were observed to be change after subjecting oil samples to deep frying for different periods.

VCO was found to experience the least change in term of fatty acid composition, while GSO generally experienced the greatest change in its fatty acid composition throughout the deep frying experiment. The change in the fatty acid composition of EVOO was generally observed to be greater than VCO but smaller than GSO throughout the deep frying experiment.

The phenomena of experiencing different degree of changes in the fatty acid compositions in different oil samples during the deep frying experiment could be due to oil samples had different percentage of heat sensitive fatty acids (unsaturated fatty acids) and different percentage of heat stable fatty acids (SFA). VCO was found to have the lowest percentages of unsaturated fatty acids (5.64%), followed by EVOO (83.77% of unsaturated fatty acids) and GSO was found to have the highest percentages of unsaturated fatty acids (89.35%). Therefore, during the deep frying experiment, having the lowest percentage of unsaturated fatty acids in VCO might prevent it to experience the most drastic change in its fatty acid composition, followed by EVOO and GSO, which had the highest percentage of unsaturated fatty acids, might allow it to experience the greatest change in its fatty acids composition. Additionally, although VCO had higher percentage of SFA than EVOO and GSO, while EVOO had higher percentage of SFA than GSO, SFA are more resistant to oxidation and degrade less readily under the conditions of deep frying. Therefore, during the deep frying experiment, the loss of SFA in VCO might not sufficient to allow it to experience a great change in its fatty acid composition as compared to EVOO and GSO, while the loss of SFA in EVOO might also not enough to produce a drastic change in its fatty acid composition as compared to GSO.

Among these three different types of vegetable oils, VCO is the most suitable for the use of domestic deep frying, followed by EVOO, and GSO is the most unsuitable for the use of domestic deep frying.

4. Conclusions

In conclusion, VCO had the greatest stability against domestic deep frying, followed by EVOO, and GSO had the least stability against domestics deep frying. In other words, among these three different types of vegetable oils, VCO is the most suitable for the use of domestic deep frying, followed by EVOO, and GSO is the most unsuitable for the use of domestic deep frying.

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Fractionation of Palm Kernel Oil by Short Path Distillation

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Abstract: Fractionation of palm kernel oil (PKO) by short path distillation (SPD) at two feed flow rates (135 g/h and 195 g/h) and six distillation temperatures, T_{Dis} (200, 210, 220, 230, 240 and 250 °C) was investigated. Other distillation parameters, such as vacuum pressure (0.001 mbar), blade rotation speed (400 rpm) and temperature of the feed material (60 °C) were kept constant. The fractionated products, known as residue and distillate, were analysed for physico-chemical properties including fatty acid composition (FAC), triacylglycerol (TAG) composition, slip melting point (SMP), thermal analysis by differential scanning calorimetry (DSC) and solid fat content (SFC). Product yield was measured as well. Crystallisation behaviour of PKO and the fractionated products were studied by measurement of isothermal crystallisation, T_{Cr} at 0, 5, 10, 15, 20 and 25 °C. The distillates, collected at all fractionation temperatures, were enriched with caprylic, capric and lauric acids. These fractions were also concentrated with low molecular weight and C36 TAGs. Distillates obtained at higher T_{Dis} (230-250 °C) exhibited higher in SMP and SFC. On the other hand, the residual oils collected at all fractionation temperatures contained higher amount of long-chain fatty acid and palmitic acid. These fractions were enriched with high molecular weight TAGs. Residues obtained at lower T_{Dis} (200-220 °C) were low in SMP and comparable SFC with PKO. Changes in fatty acid and TAG composition resulted in different crystallisation behaviour of the fractions. Distillates collected at all fractionation temperatures crystallised in a sharper peak while residues obtained at higher T_{Dis} (230-250 °C) showed broader crystallisation peaks, as shown by the DSC thermograms.

Key words: Palm kernel oil, short path distillation, palm kernel distillates, palm kernel residues, crystallisation behaviours.

1. Introduction

Oil palm fruit produces two types of oils with distinct physical and chemical properties. Palm oil is extracted from the outer mesocarp while the inner kernel of the fruits gives palm kernel oil (PKO). The former comprises medium- and long-chain fatty acids while the latter consists of short- (C6-C10), medium- (C12-C14) and long-chain (C16-C18) fatty acids. Beside coconut, babassu, tucum and ouri-curi, PKO is known as lauric oil due to its high content of lauric acid of 44% to 51%. The other major fatty acids presence includes myristic (15.3%-17.2%) and oleic acid (11.9%-18.5%) [1]. Due to considerable higher amount of oleic acid in PKO, its iodine value (IV) ranges from 16.2 to 19.6, as compared to coconut oil with IV of around 6.3 to 10.6 [2]. PKO has a wide range of triacylglycerol (TAG) compositions stretching from a total carbon number of C28 to C56, with C36 as the main TAG originated from the high lauric content. Other prominent TAGs are C34, C38, C40 and C42 [1].

High lauric acid content coupled with the wide range of TAG compositions resulted in unique melting properties of PKO. It contains high solid at lower temperatures of 5 °C to 15 °C and completely melted at 30 °C to 35 °C. It exhibited a steep melting profile.

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However, slip melting point (SMP) of PKO of around 26 °C to 28 °C is a disadvantage of the product. It is considered as too low for direct application especially as cocoa butter substitute (CBS) [3]. PKO is commonly modified.

Fractionation, interesterification, hydrogenation and simple blending are generally applied to PKO to improve its properties [3]. In normal practice, PKO is dry fractionated to produce a higher value-added product of palm kernel stearin (PKS). Palm kernel olein (PKOo) is the by-product. The PKS can be used directly as cocoa butter substitute (CBS) or further hydrogenated to improve its melting points, oxidative stabilities and crystallisation properties [4, 5]. PKS has improved solid fat profile and higher slip melting point over its native oil, which makes it suitable for confectionery fats [6].

Short path distillation (SPD) or molecular distillation is one of the oils and fats fractionation techniques. This process involves transportation of molecules from a hot surface of an evaporating component to a cool surface of a condenser in a short residence times. The process is normally conducted under very high operational vacuum levels. Applications of SPD in oils and fats include recovery of polyphenols from deodorizer distillate [7], fractionation of short and medium chain fatty acid ethyl esters [8], refining and removal of persistent organic pollutants [9], purification and deodorization of structured lipids [10], fractionation of squid visceral oil ethyl esters [11] and reduction of cholesterol in butter and lard [12]. The potential uses of SPD in fractionating milk fats proved to be quite successful in producing a desired quality of milk fat fractions [13, 14]. However, literature on physico-chemical properties of products from SPD of PKO is quite limited.

This paper investigated the fractionation of PKO by SPD under different feed flow rates and temperature conditions. Subsequently, the physico-chemical properties of the fractionated products were evaluated.

2. Materials and Methods

2.1 Materials

PKO was purchased from Pasir Gudang Edible Oil (PGEO) Sdn. Bhd., Pasir Gudang, Johor D.T., Malaysia. All chemicals used were either of analytical grade or high-performance liquid chromatography grade.

2.2 Methods

Fractionation was carried out using a laboratory short path distillation Model VKL-70-SKR-T from VTA GmbH (Deggendorf, Germany). Attached to the evaporator was a cold trap filled with liquid nitrogen to condense any volatiles and prevent it from entering the diffusion pump. A Pfeiffer rotary vane pump DUO 2.5A and diffusion pump DIF 040 L (Asslar, Germany) were used to provide the required vacuum to the evaporator.

PKO (500 g) was first heated in an oven at 60 °C for 1 h to completely melt the fat. The wiper speed and vacuum pressure of the evaporator were kept constant at 400 rpm and 0.001 mbar, respectively throughout the experiment. Two feed pump frequencies were used: 5.0 Hz and 7.5 Hz, which corresponded to feeding rates of 135 g/h and 195 g/h, respectively. Under these conditions, fractionations were performed at distillation temperatures, T_{Dis} of 200, 210, 220, 230, 240 and 250 °C. Fractionations were carried out from 30 min to 4 h, depending on the product yield. The distillate and residue were collected, weighed and stored at 10 °C prior to sample analyses.

2.3 Fatty Acid Composition (FAC)

Fatty acid methyl esters (FAMEs) were prepared using MPOB test method [15]. The oil samples were dissolved in 0.95 mL hexane in an Eppendorf tube. 50 μL sodium methoxide (1.0 M) was then added for fatty acid methylation process. After 1 h, the clear upper layer of methyl esters was pipetted off for gas chromatography analysis.

The fatty acid methyl esters was analysed by using a Hewlett-Packard 6890 series gas chromatograph, equipped with a flame ionization detector and a fused-silica capillary column (DB-23, 60 m × 0.25 mm i.d., 0.25 μm film; J&W Scientific, Folsom, CA, USA). The analysis was done according to MPOB test method [15]. The column temperature was set at 120 °C, increased to 185 °C at a rate of 3 °C/min and held for 50 min. The flow rate of helium was set at 0.8 mL/min. Injector with split ratio of 100:1 and detector temperatures were both set at 240 °C. Identification and quantification of samples were done by normalization of the composition of fatty acid methyl esters in SUPELCO RM-5 standard (Bellefonte, PA, USA).

2.4 Iodine Value (IV)

The iodine value was calculated from the composition of the unsaturated methyl esters. The calculation was based on AOCS method Cd 1c-85 [16], as follow:

Iodine value = (0.950 × % palmitic acid) + (0.860 × % oleic acid) + (1.732 × % linoleic acid) + (2.616 × % linolenic acid)

2.5 Triacylglycerol (TAG) Composition

Determination of triacylglycerol by high-performance liquid chromatography (HPLC) was done according to IUPAC method 2.323 [17]. The HPLC was equipped with a refractive index detector (Gilson, Villiersel-Bel, France) and a Lichrosphere RP18 column (250 mm × 4 mm) of 5 μm particle size (Merck, Darmstadt, Germany). Acetone/acetonitrile (70%:30% vol/vol) was used as the mobile phase at a flow rate of 1.0 mL/min. The column temperature was set at 35 °C. 20 μL sample oil, prepared at 4% concentration with acetone and chloroform as the solvent, was injected. Identification of the TAGs was made by comparison with those of available standards, purchased through Sigma Chemical Co. (St-Louis, MO, USA). The TAGs were expressed as total carbon number (CN) in weight percentage of the total TAGs. Table 1 tabulates the FAC of triacylglycerol groups of PKO according to the CN.

2.6 Solid Fat Content (% SFC) by Nuclear Magnetic Resonance (NMR)

The oil samples were melted and filled into the sample tubes (10 mm o.d. × 75 mm length) up to a 3 cm height. Then they were tempered at 70 °C for 30 min, chilled at 0 °C for 90 min, and kept at the desired temperatures in pre-equilibrated thermostated baths for 30 min prior to measurement. The measuring temperatures were 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 °C. The instrument used was a Bruker Minispec PC 120 pulsed NMR. The direct method was employed for the measurements.

Isothermal crystallization, T_{Cr}, was characterized at 5, 10, 15, 20, 25 and 30 °C. Samples were first melted in

Table 1 Fatty acid composition of triacylglycerol groups of PKO.

CN	Groups*	Types#
30	*SSS*	C6C12C12
		C8C10C12
32	*SSS*	C8C12C12
34	*SSS*	C8C12C14
		C10C12C12
36	*SSS*	C12C12C12
38	*SSS*	C12C12C14
40	*SSS*	C12C12C16
		C12C14C14
42	*SSU*	C12C12**C18**
	SSS	C12C14C16
44	*SSU*	C12C14**C18**
	SSS	C12C16C16
46	*SSU*	C12C16**C18**
		C14C14**C18**
48	*SUU*	C12**C18C18**
	SSU	C14C16**C18**
	SSS	C16C16C16
50	*SUU*	C14**C18C18**
	SSU	C16C16**C18**
	SSS	C16C16C18
52	*SUU*	C16**C18C18**
	SSU	C16C18**C18**
54	*UUU*	**C18C18C18**
	SUU	C18**C18C18**
	SSU	C18C18**C18**

**SSS*: trisaturated; *SSU*: monounsaturated; *SUU*: diunsaturated; *UUU*: triunsaturated TAGs.

#C6: caproic; C8: caprylic; C10: capric; C12: lauric; C14: myristic; C16: palmitic; C18: stearic; **C18: oleic** acids.

NMR tubes at 70 °C for 30 min. The iso-solid was measured after the samples were placed at the required temperatures at appropriate time intervals.

2.7 Slip Melting Point (SMP)

Slip melting point was determined by the AOCS method Cc 3-25 [16]. Six capillary tubes were each filled with a 10-mm-high column of fat. The fat column was chilled by holding and rolling the ends of the tubes containing the sample pressed against a piece of ice until the fat solidified. The tubes were placed in a test tube and held in a beaker of water equilibrated at 10 ± 1 °C in a thermostat water bath. The beaker was transferred to the water bath and held for 16 h at 10 ± 1 °C. The capillary tubes were then removed from the test tube and attached to a thermometer with a rubber band such that the lower ends of the tubes were level with the bottom of the mercury bulb of the thermometer. After that, the thermometer was suspended in a beaker containing 400 mL boiled distilled water such that the lower end of the thermometer was immersed in the water to a depth of 30 mm. The starting temperature of the bath was adjusted to 8 °C to 10 °C below the expected SMP of the sample. The water was agitated with a magnetic stirrer and heat was applied to increase the temperature at a rate of 1 °C/min, slowing down to 0.5 °C/min as the slip point was reached. The heating was continued until the fat column was raised. The temperature at which the fat column rose was reported as SMP.

2.8 Thermal Analysis-Differential Scanning Calorimetry

Differential scanning calorimetry analysis was performed with a Perkin Elmer DSC-7 (Norwalk, CT, USA). The instrument was calibrated with indium by a temperature programme of 120 °C to 180 °C at a rate of 5 °C/min.

Prior to weighing (10 mg) each sample was completely melted at 80 °C into an aluminium pan. It was then sealed using a sample pan crimper. The previous thermal history of the sample was erased by heating to 80 °C in the DSC instrument. The samples were cooled to -50 °C at a rate of -5 °C/min and held at that temperature for 10 min. The samples were then heated to 80 °C at a rate of 5 °C/min. The melting and cooling thermograms were recorded.

2.9 Statistical Analysis

Data obtained from the measurements were subjected to one-way analysis of variance (one-way ANOVA) with Tukey's multiple comparison to determine the significant differences among the samples defined at 95% confidence interval ($P < 0.05$). All measurements were conducted in duplicate and reported as means ± standard deviation (SD) of independent trials. The statistical analyses were performed using the Minitab software Version 16 statistical package (Minitab Inc., State College, PA, USA).

3. Results and Discussion

Factors influencing separation by SPD include operating pressure (vacuum), temperature of feed material, wiper speed, feeding rate and evaporation temperature. In this study, the first three factors were kept constant. The effect of feeding rate and evaporation temperature, T_{Dis}, on the yield was investigated. Yield of distillate increases at higher T_{Dis} and decreases at higher feeding rates [7]. Fig. 1 illustrates the effect of feeding rate and T_{Dis} on the product yield. Distillate yields increase as T_{Dis} increases. At T_{Dis} of 200 °C, less than 20% of distillates were collected whereas at 250 °C, the total distillates were more than 80%. It was also observed that higher yields were obtained at a lower feeding rate (135 g/h) as compared to a higher (195 g/h) feeding rate. As shown by Fig. 1, lower feeding rate resulted in better separation and hence, products (distillates and residues) collected from these conditions were further examined.

3.1 Fatty Acid Composition

Fractionation of PKO by SPD significantly ($P < 0.05$)

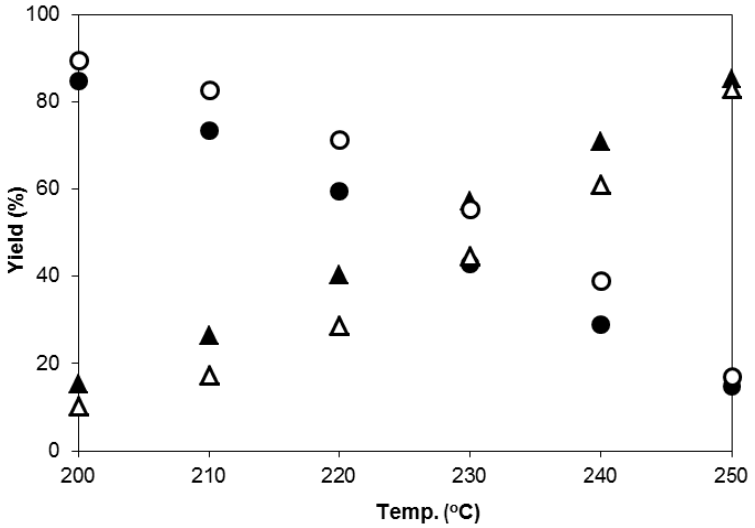


Fig. 1 Effect of feeding rate and evaporation temperature on product yield.

▲ : Distillate (135 g/h) ● : Residue (135 g/h)

△ : Distillate (195 g/h) ○ : Residue (195 g/h)

altered the fatty acid composition of the original oil. Table 2 tabulates the FAC of PKO and the distillates obtained at different fractionation temperatures. A significantly ($P < 0.05$) higher concentration of caprylic, capric and lauric acids, and a lower concentration of long-chain fatty acids was observed in the distillates obtained at all fractionation temperatures relative to the original PKO. Distillate obtained at 200 °C showed the highest content of caprylic and capric acids and at the same time exhibited the lowest amount of myristic, palmitic and oleic acids, which is a favourable for the medium chain triglyceride (MCT) application. The least amount of oleic acid in the distillates collected at all fractionation temperatures resulted in a significantly ($P < 0.05$) lower iodine value (IV) of the fraction.

Long-chain fatty acids as well as palmitic acid was significantly ($P < 0.05$) concentrated in the residues as illustrated in Table 3. The caprylic, capric and lauric acids were significantly ($P < 0.05$) depleted at all fractionation temperatures. Residue obtained at 250 °C showed the highest content of long-chain fatty acids and the lowest amount of medium- and short-chain fatty acids. A significantly ($P < 0.05$) higher amount of oleic acid in the residues resulted in significantly ($P < 0.05$) higher IV of the fraction. The residues especially collected at 250 °C are suitable for use as an oleic acid source for the oleochemical industry.

3.2 Triacylglycerol Composition

Tables 4 and 5 summarize the TAG composition of PKO and the fractionated products obtained at different fractionation temperatures. The low molecular weight (C30-C34) and C36 TAGs were significantly ($P < 0.05$) concentrated in the distillates, while the high molecular weight (C42-C54) TAGs were enriched in the residues. As compared to PKO, composition of the C38 TAGs in the distillates was significantly ($P < 0.05$) lower at fractionation temperatures below 230 °C. As the temperature increases (≥ 230 °C), its composition significantly ($P < 0.05$) increases to higher than that of PKO. Generally, it was observed that the distillates are "purer" in composition, having mainly low and medium (C36-C40) molecular weights TAGs. Whilst, the residues contain a well distributed range of different molecular weights TAGs.

3.3 Slip Melting Point

The slip melting point of the fractionated products collected at different fractionation temperatures

Table 2 Fatty acid composition of PKO and distillates at different distillation temperatures and feeding rate of 135 g/h.

Fatty acid	PKO	Distillation temperature (°C)					
		200	210	220	230	240	250
C8:0	4.5 ± 0.1^{a}	12.5 ± 0.1^{b}	11.1 ± 0.1^{c}	9.3 ± 0.1^{d}	7.7 ± 0.1^{e}	6.4 ± 0.1^{f}	5.7 ± 0.1^{g}
C10:0	3.8 ± 0.1^{a}	7.2 ± 0.1^{b}	7.0 ± 0.1^{b}	6.5 ± 0.1^{c}	5.9 ± 0.1^{d}	5.1 ± 0.1^{e}	4.6 ± 0.1^{f}
C12:0	49.8 ± 1.0^{a}	64.1 ± 0.1^{b}	64.6 ± 0.1^{b}	64.7 ± 0.2^{b}	64.0 ± 0.2^{b}	61.6 ± 0.1^{c}	58.4 ± 0.2^{d}
C14:0	15.8 ± 0.1^{a}	10.5 ± 0.1^{b}	11.3 ± 0.0^{c}	12.7 ± 0.1^{d}	14.0 ± 0.0^{e}	15.6 ± 0.1^{a}	16.4 ± 0.1^{f}
C16:0	7.7 ± 0.1^{a}	2.0 ± 0.0^{b}	2.3 ± 0.0^{c}	2.8 ± 0.0^{d}	3.5 ± 0.1^{e}	4.6 ± 0.1^{f}	5.7 ± 0.1^{g}
C18:0	2.0 ± 0.1^{a}	0.5 ± 0.2^{b}	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}	0.7 ± 0.1^{bc}	0.9 ± 0.0^{cd}	1.3 ± 0.1^{d}
C18:1	13.8 ± 0.8^{a}	2.0 ± 0.1^{b}	2.2 ± 0.0^{b}	2.8 ± 0.1^{bc}	3.5 ± 0.1^{c}	5.0 ± 0.1^{d}	7.0 ± 0.1^{e}
C18:2	2.3 ± 0.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	0.3 ± 0.1^{bc}	0.4 ± 0.1^{bc}	0.6 ± 0.1^{c}	0.8 ± 0.1^{d}
IV	15.8 ± 1.2^{a}	2.1 ± 0.1^{b}	2.2 ± 0.0^{b}	2.9 ± 0.1^{b}	3.7 ± 0.1^{bc}	5.2 ± 0.0^{c}	7.4 ± 0.1^{d}

Mean of duplicate analysis ± SD.
Values within a row with different superscript letters are significantly different ($P < 0.05$).

Table 3 Fatty acid composition of PKO and residues at different distillation temperatures and feeding rate of 135 g/h.

Fatty acid	PKO	Distillation temperature (°C)					
		200	210	220	230	240	250
C8:0	4.5 ± 0.1^{a}	3.6 ± 0.0^{b}	3.0 ± 0.0^{c}	2.4 ± 0.0^{d}	1.9 ± 0.0^{e}	1.5 ± 0.0^{f}	1.0 ± 0.0^{g}
C10:0	3.8 ± 0.1^{a}	3.4 ± 0.1^{b}	3.0 ± 0.1^{c}	2.6 ± 0.1^{d}	2.0 ± 0.1^{e}	1.5 ± 0.1^{f}	1.0 ± 0.0^{g}
C12:0	49.8 ± 1.0^{a}	47.9 ± 0.0^{a}	46.4 ± 0.0^{b}	43.1 ± 0.1^{c}	37.3 ± 0.1^{d}	29.7 ± 0.0^{e}	21.6 ± 0.1^{f}
C14:0	15.8 ± 0.1^{a}	16.3 ± 0.1^{b}	16.7 ± 0.1^{c}	17.1 ± 0.1^{d}	17.1 ± 0.1^{d}	15.8 ± 0.1^{a}	13.3 ± 0.1^{e}
C16:0	7.7 ± 0.1^{a}	8.4 ± 0.1^{b}	9.0 ± 0.1^{c}	9.9 ± 0.0^{d}	11.4 ± 0.1^{e}	13.2 ± 0.0^{f}	14.7 ± 0.1^{g}
C18:0	2.0 ± 0.1^{a}	2.3 ± 0.0^{ab}	2.4 ± 0.1^{ab}	2.8 ± 0.1^{bc}	3.3 ± 0.1^{c}	4.2 ± 0.0^{d}	4.8 ± 0.2^{e}
C18:1	13.8 ± 0.8^{a}	15.3 ± 0.0^{a}	16.5 ± 0.1^{b}	18.8 ± 0.1^{c}	22.9 ± 0.2^{d}	28.7 ± 0.0^{e}	36.4 ± 0.1^{f}
C18:2	2.3 ± 0.1^{a}	2.5 ± 0.1^{a}	2.8 ± 0.1^{b}	3.2 ± 0.1^{c}	4.0 ± 0.1^{d}	5.1 ± 0.0^{e}	7.0 ± 0.0^{f}
IV	15.8 ± 1.2^{a}	17.5 ± 0.0^{ab}	18.9 ± 0.3^{b}	21.6 ± 0.2^{c}	26.5 ± 0.4^{d}	33.5 ± 0.0^{e}	43.3 ± 0.0^{f}

Mean of duplicate analysis ± SD.
Values within a row with different superscript letters are significantly different ($P < 0.05$).

Table 4 Triacylglycerol composition of PKO and distillates at different distillation temperatures and feeding rate of 135 g/h.

TAGs (CN)	PKO	Distillation temperature (°C)					
		200	210	220	230	240	250
C30	1.5 ± 0.1^{a}	9.0 ± 0.1^{b}	6.8 ± 0.1^{c}	4.6 ± 0.1^{d}	3.3 ± 0.1^{e}	2.3 ± 0.0^{f}	2.1 ± 0.3^{f}
C32	6.1 ± 0.1^{a}	26.5 ± 0.2^{b}	23.6 ± 0.1^{c}	18.2 ± 0.1^{d}	13.8 ± 0.1^{e}	10.1 ± 0.1^{f}	8.2 ± 0.0^{g}
C34	8.9 ± 0.1^{a}	19.4 ± 0.1^{b}	20.4 ± 0.1^{c}	19.7 ± 0.1^{d}	17.4 ± 0.1^{e}	14.1 ± 0.0^{f}	11.8 ± 0.0^{g}
C36	21.4 ± 0.0^{a}	24.1 ± 0.0^{b}	28.4 ± 0.1^{c}	32.8 ± 0.3^{d}	34.4 ± 0.4^{e}	32.4 ± 0.2^{d}	28.4 ± 0.1^{c}
C38	16.9 ± 0.1^{a}	9.4 ± 0.1^{b}	11.1 ± 0.0^{c}	14.3 ± 0.1^{d}	17.8 ± 0.0^{e}	21.0 ± 0.1^{f}	21.0 ± 0.0^{f}
C40	9.6 ± 0.0^{a}	2.1 ± 0.1^{b}	2.8 ± 0.1^{c}	4.0 ± 0.0^{d}	5.8 ± 0.1^{e}	8.5 ± 0.0^{f}	10.2 ± 0.0^{g}
C42	9.9 ± 0.0^{a}	1.1 ± 0.1^{b}	1.4 ± 0.0^{c}	2.2 ± 0.1^{d}	3.5 ± 0.1^{e}	5.9 ± 0.1^{f}	8.6 ± 0.1^{g}
C44	7.3 ± 0.0^{a}	0.4 ± 0.1^{b}	0.5 ± 0.0^{bc}	0.8 ± 0.1^{c}	1.3 ± 0.1^{d}	2.4 ± 0.1^{e}	4.2 ± 0.0^{f}
C46	4.4 ± 0.0^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{c}	0.3 ± 0.0^{d}	0.5 ± 0.0^{e}	1.0 ± 0.0^{f}	2.0 ± 0.0^{g}
C48	6.0 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.2 ± 0.0^{c}	0.3 ± 0.0^{d}	0.7 ± 0.1^{e}	1.6 ± 0.0^{f}
C50	3.0 ± 0.0^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.1 ± 0.0^{b}	0.4 ± 0.0^{c}
C52	2.1 ± 0.1^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.2 ± 0.0^{b}
C54	2.3 ± 0.1^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.0^{b}

Mean of duplicate analysis ± SD.
Values within a row with different superscript letters are significantly different ($P < 0.05$).

is shown in Fig. 2. The SMP of distillates increased with increasing T_{Dis}, whereas the SMP of residues decreased with increasing T_{Dis}. The variation in SMP is related to the chemical composition (FA and TAG composition) of the fractionated products.

Two distinct regions of SMP were observed in Fig. 2. At T_{Dis} below 220 °C, the SMP of the residues was higher than distillates. However, the SMP of the residues were lower than distillates at T_{Dis} higher than 220 °C. The SMP of both fractions at T_{Dis} 220 °C were found to be approximately similar to PKO. At T_{Dis} below 220 °C, the residues were examined to have a

Table 5 Triacylglycerol composition of PKO and residues at different distillation temperatures and feeding rate of 135 g/h.

TAGs (CN)	PKO	Distillation temperature (°C)					
		200	210	220	230	240	250
C30	1.5 ± 0.1[a]	0.6 ± 0.1[b]	0.3 ± 0.0[c]	0.0 ± 0.0[d]	0.0 ± 0.0[d]	0.0 ± 0.0[d]	0.0 ± 0.0[d]
C32	6.0 ± 0.1[a]	3.8 ± 0.0[b]	2.5 ± 0.0[c]	1.3 ± 0.0[d]	0.4 ± 0.0[e]	0.2 ± 0.0[f]	0.1 ± 0.0[g]
C34	8.9 ± 0.1[a]	7.7 ± 0.0[b]	6.5 ± 0.0[c]	4.6 ± 0.1[d]	2.3 ± 0.3[e]	1.2 ± 0.0[f]	0.5 ± 0.1[g]
C36	21.4 ± 0.0[a]	21.1 ± 0.1[ab]	20.0 ± 0.0[b]	17.3 ± 0.6[c]	11.1 ± 0.4[d]	5.2 ± 0.0[e]	1.7 ± 0.0[f]
C38	16.1 ± 0.1[a]	17.6 ± 0.0[ab]	18.0 ± 0.0[b]	18.2 ± 0.6[b]	15.8 ± 0.4[c]	10.6 ± 0.0[d]	5.2 ± 0.1[e]
C40	9.6 ± 0.0[a]	10.5 ± 0.0[b]	11.1 ± 0.0[c]	11.9 ± 0.0[d]	12.6 ± 0.2[e]	11.4 ± 0.1[c]	7.4 ± 0.0[f]
C42	9.9 ± 0.0[a]	10.9 ± 0.1[ab]	11.7 ± 0.1[bc]	12.8 ± 0.8[c]	14.9 ± 0.1[de]	15.7 ± 0.0[e]	14.1 ± 0.1[d]
C44	7.3 ± 0.0[a]	8.1 ± 0.1[ab]	8.6 ± 0.0[b]	9.5 ± 0.6[c]	12.1 ± 0.0[d]	14.3 ± 0.0[e]	15.7 ± 0.1[f]
C46	4.4 ± 0.0[a]	4.8 ± 0.0[b]	5.2 ± 0.0[c]	6.0 ± 0.1[d]	7.5 ± 0.0[e]	9.3 ± 0.0[f]	11.1 ± 0.1[g]
C48	6.0 ± 0.0[a]	6.6 ± 0.1[ab]	7.1 ± 0.2[bc]	7.8 ± 0.4[d]	10.6 ± 0.0[e]	13.8 ± 0.1[f]	18.2 ± 0.1[g]
C50	3.0 ± 0.0[a]	3.4 ± 0.1[ab]	3.6 ± 0.0[b]	4.2 ± 0.1[c]	5.7 ± 0.3[d]	7.3 ± 0.1[e]	10.3 ± 0.1[f]
C52	2.1 ± 0.1[a]	2.4 ± 0.1[a]	2.6 ± 0.1[a]	3.1 ± 0.1[a]	2.9 ± 1.4[a]	5.3 ± 0.1[b]	7.6 ± 0.0[c]
C54	2.4 ± 0.1[a]	2.7 ± 0.1[a]	2.9 ± 0.1[ab]	3.4 ± 0.3[b]	4.2 ± 0.4[c]	5.9 ± 0.1[d]	8.5 ± 0.1[e]

Mean of duplicate analysis ± SD.

Values within a row with different superscript letters are significantly different ($P < 0.05$).

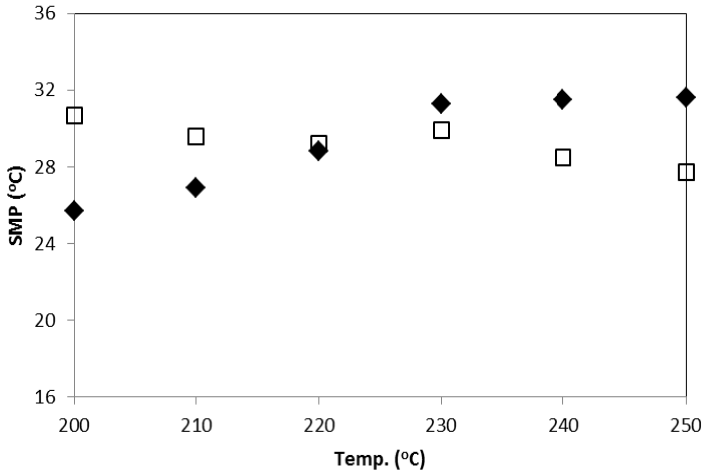


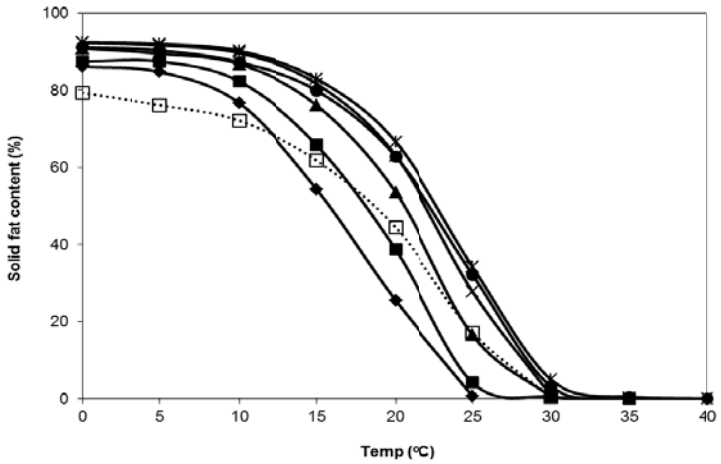
Fig. 2 Slip melting point of distillates (♦) and residues (□) obtained at different distillation temperatures and feeding rate of 135 g/h. Slip melting point of PKO is 29.6 °C.

significantly ($P < 0.05$) higher amount of medium molecular weight (C36-C40) TAGs than PKO. As such, these residues were higher in SMP. At T_{Dis} above 220 °C, the medium molecular weight TAGs in the residues were significantly ($P < 0.05$) lower, which subsequently decreased the SMP of residues.

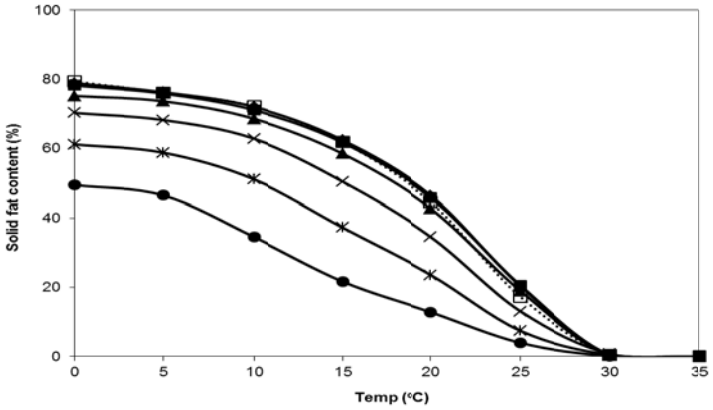
On the other hand, distillates contained a significantly ($P < 0.05$) lower amount of medium molecular weight TAGs at T_{Dis} below 220 °C than PKO, hence lower values of SMP were observed. Distillates at T_{Dis} above 220 °C, which possessed higher amount of medium molecular weight TAGs, resulted in higher SMP. Low and high molecular weight TAGs were not significantly ($P \geq 0.05$) contribute to the SMP trend demonstrated in Fig. 2. Distillates contained higher amount of the former but lower content of the latter at all T_{Dis} as compared to PKO.

3.4 Solid Fat Content Profile

Figs. 3a and 3b illustrate the solid fat content profile of the distillates and residues as compared to PKO. In line with their chemical compositions, the distillates collected at all fractionation temperatures contained higher solid fat than that of PKO at temperature range of 0 °C to 10 °C. At temperature range of 20 °C to 25 °C, distillates obtained at lower fractionation



(a)



(b)

Fig. 3 Solid fat content (%) vs. temperature (°C) for (a) distillates and (b) residues obtained at 200 °C (♦), 210 °C (■), 220 °C (▲), 230 °C (×), 240 °C (✳), 250 °C (●) and palm kernel oil (□).

temperatures (T_{Dis} < 220 °C) exhibited lower solid fat and had steeper slopes than PKO. PKO and the distillates completely melted at temperature below 35 °C.

The SFC curves of the residues are rather flatter as compared to the distillates. The SFC values of the residues collected at T_{Dis} of below 230 °C were comparable to PKO while the rest of the fractions exhibited lower SFC values at all temperatures. All residues completely melted at temperature 30 °C.

3.5 Crystallisation Behaviour

Crystallisation behaviour of PKO and the fractions obtained at different T_{Cr} is shown in Figs. 4 and 5. The crystallisation curves are, in general, sigmoidal in shape, an indication of nucleation followed by crystal growth of the crystallisation process [18-20]. Maximum solid fat content (SFC_{max}) was achieved rapidly at lower T_{Cr}. A higher T_{Cr} resulted in less solid fat content [21].

In general, the SFC_{max} of distillates is higher than PKO. The crystallisation rate is almost similar except for the distillate obtained at 200 °C, where it crystallised at a slower rate. At T_{Cr} of 10 °C, only distillates obtained at higher T_{Dis} (210-250 °C) exhibited sigmoidal crystallisation curve with SFC_{max} higher than PKO. At the highest T_{Cr}, i.e., 25 °C (Fig. 4f), all samples (distillates and PKO) were found not to demonstrate sigmoidal trend.

The sigmoidal shape of crystallisation curve was also observed for residues, especially at T_{Cr} of 0 °C to 15 °C (Figs. 5a-5d) but not at higher T_{Cr} of 20 °C to

25 °C (Figs. 5e and 5f). Most of the residues had a comparable or lower SFC_{max} than PKO. Maximum SFC was also achieved quickly at lower T_{Cr}. The crystallisation rates were decreased as the T_{Cr} increased.

According to Marangoni [22], fats usually have to be undercooled by at least 5-10 °C below its melting point before they begin to crystallise. For a few degrees below the melting point (lower supercooling), metastable region exists. Melting point of PKO and the fractionated products was ranged from 27.7 °C to 31.6 °C. As such, a fractionation product with melting point of 27.7 °C exists in the metastable region when it is crystallised at 25 °C. In this region, the sigmoidal trend is not observed.

3.6 Thermal Analysis by Differential Scanning Calorimetry

The thermograms of PKO, distillates and residues

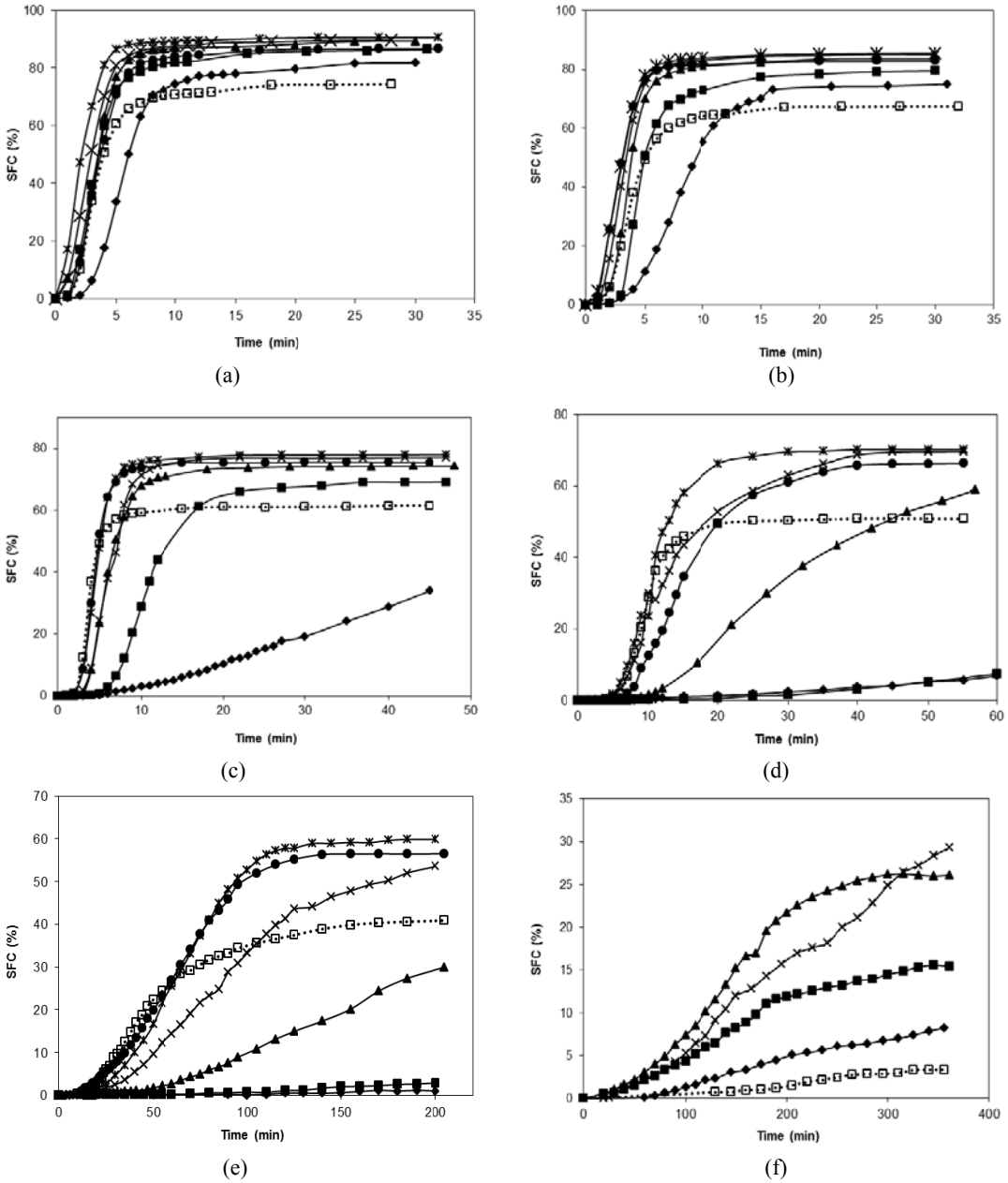


Fig. 4 Solid fat content (%) vs. time (min) during crystallisation at (a) 0 °C, (b) 5 °C, (c) 10 °C, (d) 15 °C, (e) 20 °C and (F) 25 °C of distillates obtained at 200 °C (♦), 210 °C (■), 220 °C (▲), 230 °C (×), 240 °C (✳), 250 °C (●) and palm kernel oil (□).

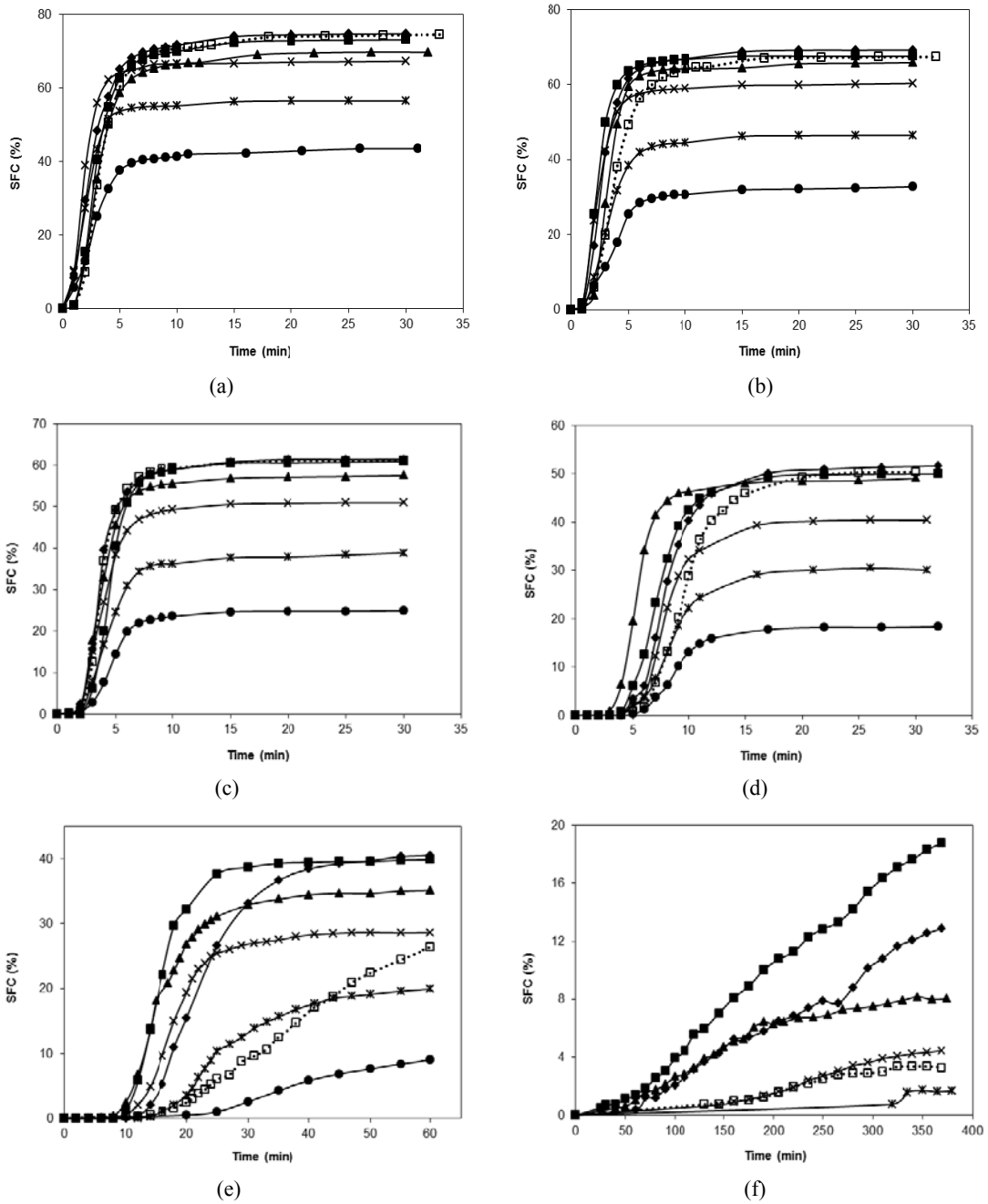


Fig. 5 Solid fat content (%) vs. time (min) during crystallisation at (a) 0 °C, (b) 5 °C, (c) 10 °C, (d) 15 °C, (e) 20 °C and (f) 25 °C of residues obtained at 200 °C (♦), 210 °C (■), 220 °C (▲), 230 °C (×), 240 °C (✳), 250 °C (●) and palm kernel oil (□).

are shown in Figs. 6 and 7. Cooling thermogram of PKO shows a broad peak with a shoulder at 3.3 °C and 6.3 °C. The melting endotherms of PKO are made up of four peaks representing four TAG groups of different melting points. Tables 6 and 7 tabulate the four TAG groups, namely *UUU*, *SUU*, *SSU* and *SSS*. The first and second minor endotherms at -19.1 °C and 0.1 °C represent the *UUU* and *SUU* TAGs, respectively. The two major endotherms at 14.3 °C and 26.7 °C (Table 8) demonstrate the *SSU* and *SSS* TAGs, respectively. PKO was found to melt completely at 28.8 °C.

As shown in Fig. 6, fractionation by SPD resulted in sharper crystallisation peaks of distillates with increase intensity as compared to PKO. This was due to the

Table 6 Distribution of trisaturated, monounsaturated, diunsaturated and triunsaturated triacylglycerols in PKO and distillates.

Sample	TAG distribution (%)			
	SSS	*SSU*	*SUU*	*UUU*
PKO	71.4 ± 0.1^{a}	18.1 ± 0.1^{a}	7.8 ± 0.1^{a}	1.9 ± 0.1^{a}
DIS200	90.9 ± 0.3^{b}	1.0 ± 0.0^{b}	0.1 ± 0.0^{b}	0.0 ± 0.0^{b}
DIS210	93.6 ± 0.2^{c}	1.4 ± 0.0^{c}	0.1 ± 0.0^{b}	0.0 ± 0.0^{b}
DIS220	94.5 ± 0.0^{d}	2.1 ± 0.0^{d}	0.1 ± 0.0^{b}	0.0 ± 0.0^{b}
DIS230	94.1 ± 0.3^{cd}	3.6 ± 0.2^{e}	0.2 ± 0.0^{c}	0.0 ± 0.0^{b}
DIS240	91.4 ± 0.2^{b}	6.4 ± 0.1^{f}	0.6 ± 0.1^{d}	0.2 ± 0.0^{c}
DIS250	86.4 ± 0.1^{e}	10.6 ± 0.0^{g}	1.4 ± 0.0^{e}	0.3 ± 0.0^{d}

Mean of duplicate analysis ± SD.

Values within a column with different superscript letters are significantly different ($P < 0.05$).

Table 7 Distribution of trisaturated, monounsaturated, diunsaturated and triunsaturated triacylglycerols in PKO and residues.

Sample	TAG distribution (%)			
	SSS	*SSU*	*SUU*	*UUU*
PKO	71.4 ± 0.1^{a}	18.1 ± 0.1^{a}	7.8 ± 0.1^{a}	1.9 ± 0.1^{a}
RES200	69.3 ± 0.0^{ab}	20.0 ± 0.1^{ab}	8.5 ± 0.1^{b}	2.1 ± 0.0^{a}
RES210	67.0 ± 0.2^{b}	21.5 ± 0.1^{b}	9.2 ± 0.2^{b}	2.3 ± 0.1^{a}
RES220	62.6 ± 0.6^{c}	24.2 ± 0.2^{c}	10.4 ± 0.4^{c}	2.7 ± 0.1^{b}
RES230	54.0 ± 1.6^{d}	28.5 ± 1.6^{d}	14.1 ± 0.2^{d}	3.4 ± 0.2^{c}
RES240	41.9 ± 0.1^{e}	35.3 ± 0.1^{e}	18.4 ± 0.1^{e}	4.5 ± 0.0^{d}
RES250	28.7 ± 0.1^{f}	39.5 ± 0.0^{f}	25.4 ± 0.0^{f}	6.4 ± 0.1^{e}

Mean of duplicate analysis ± SD.

Values within a column with different superscript letters are significantly different ($P < 0.05$).

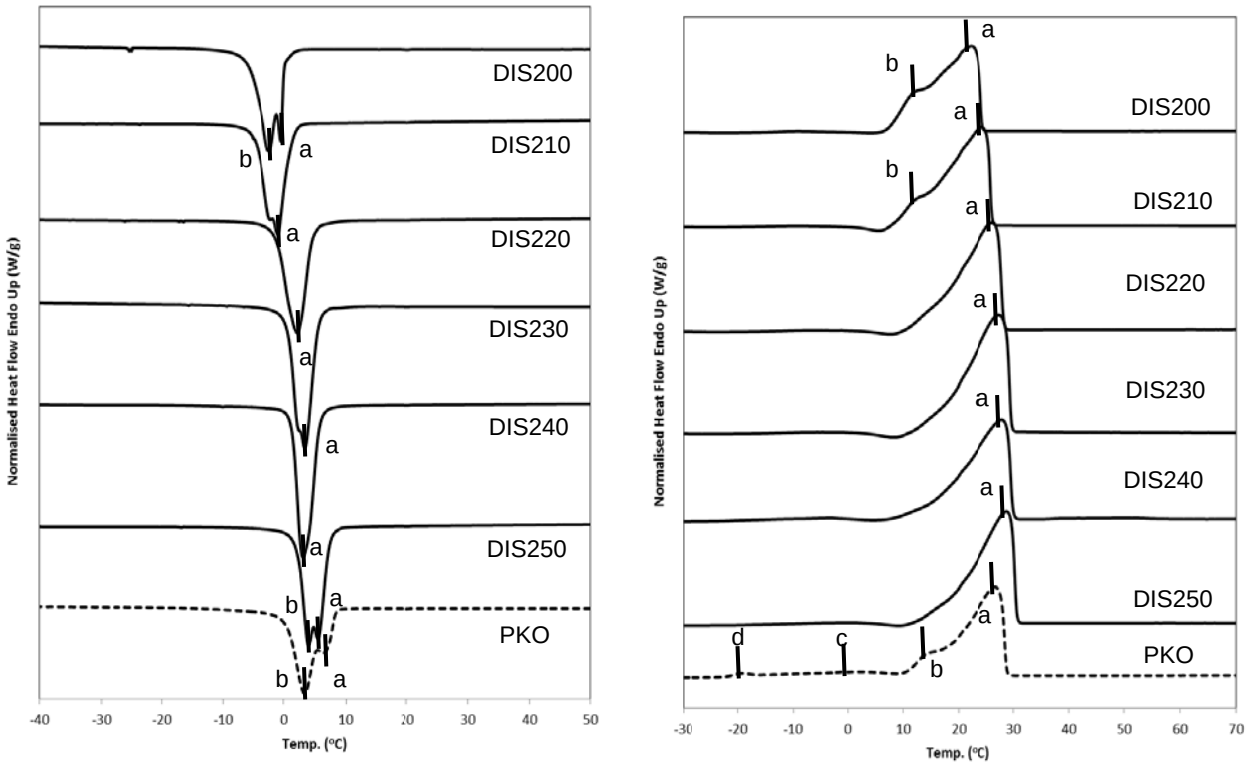


Fig. 6 Cooling and melting thermograms of PKO and distillates.

Table 8 Cooling and melting peaks of PKO and distillates.

Sample	Cooling thermograms (°C)		Melting thermograms (°C)			
	a	b	a	b	c	d
DIS200	-0.5	-2.5	22.2	12.4	-	-
DIS210	-1.0	-	24.2	12.6	-	-
DIS220	2.1	-	26.1	-	-	-
DIS230	3.4	-	27.4	-	-	-
DIS240	3.1	-	27.9	-	-	-
DIS250	5.5	4.0	28.7	-	-	-
PKO	6.3	3.3	26.7	14.3	0.1	-19.1

concentrated amount of trisaturated *SSS* TAGs as illustrated in Table 6. Sharper peak is an indicative of a sharper melting point of the distillates. Siew [23] reported that the trisaturated trilaurin C36 TAG (LaLaLa) crystallised in a sharp peak at 10.7 °C with a shoulder at 12 °C. Even though the distillates contained significant amount of trilaurin, the exotherm peaks shifted to a lower temperature due to the presence of relatively lower melting TAGs (Table 6). Fractionation resulted in elimination of the two minor endotherms at lower temperatures. This is due to the absence of the low melting point TAGs in the distillates as shown in Table 6. In general, the shape of the endotherms is almost similar to that of PKO. Distillates obtained at T_{Dis} of 200 °C to 220 °C had the broad peak shifted to lower temperatures whereas distillates collected at T_{Dis} of 230 °C and above had the broad peak moved to higher temperatures as compared to PKO.

Cooling thermograms of the residues are shown in Fig. 7 and the peaks are illustrated in Table 9. The residues at lower T_{Dis} (200-220 °C) exhibited similar pattern to that of PKO. As the T_{Dis} increases, the *SSS* TAGs were significantly reduced while the *SUU* and *UUU* TAGs were enriched (Table 7). The progressive reduction of *SSS* TAGs and enrichment of *SUU* and *UUU* was translated into broader peaks of the

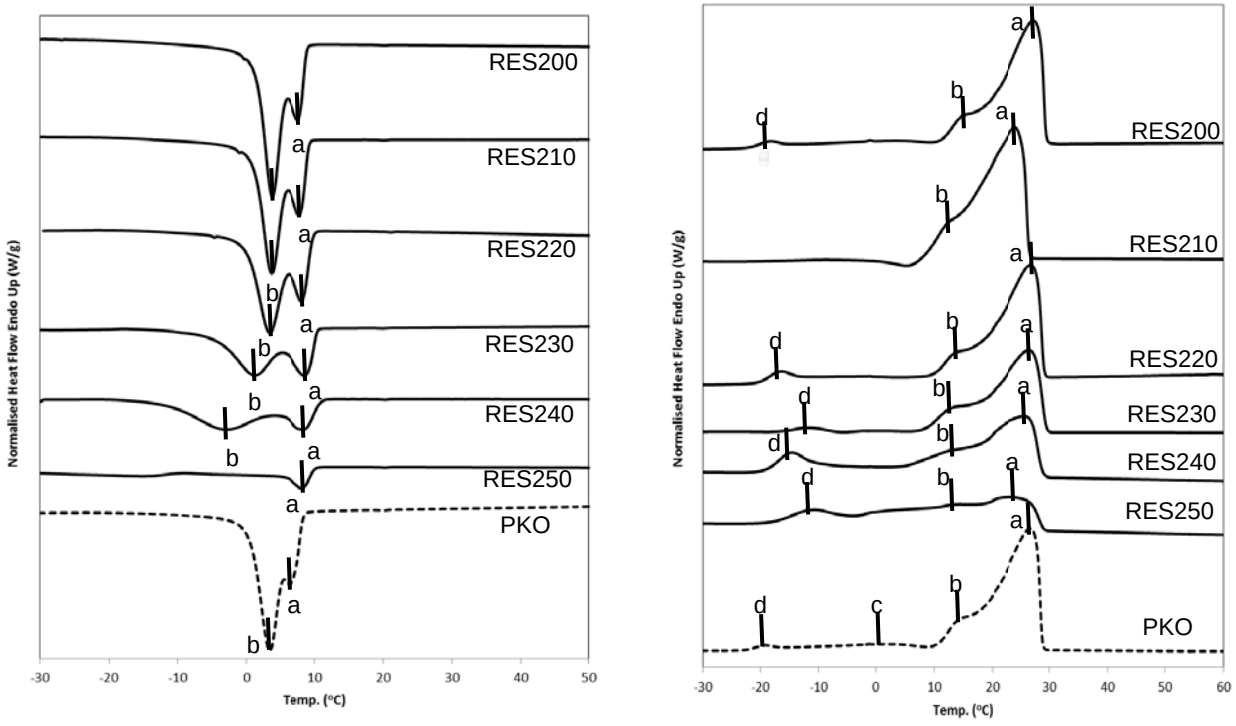


Fig. 7 Cooling and melting thermograms of PKO and residues.

Table 9 Cooling and melting peaks of PKO and residues.

Sample	Cooling thermograms (°C)		Melting thermograms (°C)			
	a	b	a	b	c	d
RES200	7.5	3.7	27.2	14.8	-	-18.2
RES210	7.8	3.6	24.1	12.5	-	-
RES220	8.1	3.3	26.7	13.9	-	-16.4
RES230	8.5	1.2	26.4	13.3	-	-11.7
RES240	8.4	-3.0	25.5	13.1	-	-14.6
RES250	8.1	-	23.8	14.1	-	-10.8
PKO	6.3	3.3	26.7	14.3	0.1	-19.1

residues collected at T_{Dis} of 230 °C and above. The intensity of the peaks was reduced as well. Similarly, residues obtained at lower T_{Dis} (200-220 °C) exhibited almost similar endotherm as PKO. Low melting endotherms were present in the residues due to the concentrated lower melting TAGs of *SUU* and *UUU* (Table 7). At higher T_{Dis} (230-250 °C), broader endotherms were well observed due to wider range of TAGs in the residues.

4. Conclusions

Palm kernel oil was fractionated by short path distillation to yield distillates and residues. These fractions were distinctly different in their physico-chemical properties. Distillate especially collected at the highest T_{Dis} (250 °C) was high in SMP, SFC and enriched with caprylic, capric and lauric acids. It also concentrated with low molecular weight and C36 TAGs. The fraction crystallised in a sharper peak than PKO. On the other hand, the residue particularly obtained at the lowest T_{Dis} (200 °C) was low in SMP, comparable SFC with PKO and enhanced with long-chain and palmitic acid. The high molecular weight TAGs were increased in this fraction.

Acknowledgments

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Conservation of Leaves of a Medicinal Plant of Western Algeria (*Pistacia atlantica*)

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Abstract: In our context, and in the goal to valorize the *Pistacia atlantica* species Desf that grows spontaneously in Algeria occidental except the coastline, and that is used by the nomadic populations in their daily consumptions, we are thinking about drying its leaves. Here, the biochemical analysis of dried leaves of *Pistacia atlantica* is determined, the sorption is other ms are of great importance in the food industry, especially in the drying; the sorption isotherms of pistachio leaves were measured by the gravimetric method at three temperatures 40, 50 and 60 °C. The equilibrium was achieved after eight days for desorption and seven days for adsorption with water activity ranging from 5% to 90%. Only the GAB and Peleg models were found to be the most suitable for describing the sorption curves. The isosteric heat of sorption of *Pistacia atlantica* leaves decreases with an increase in moisture content and was found to be an exponential function of moisture content for adsorption and desorption. The pistachio leaves could be considered as a rich natural source of valuable nutriments (carbohydrates, proteins and lipids); lipid fraction is equal to 2.25%; proteins are the second macronutriment that predominates in these sheets: 4.35%; accordingly carbohydrates content was about 25.77%.

Key words: *Pistacia atlantica* leaves, equilibrium moisture content, sorption isotherms, modeling, isosteric heat of sorption biochemical analysis.

Nomenclature

ads	Adsorption
Aw	Water activity
d.b	Dry basis
des	Desorption
df	Number of degrees of freedom
$X_{e\,q}$	Equilibrium moisture content (% d.b.)
$X_{i;exp}$	Ith experimental moisture content (% d.b.)
$X_{i;pre}$	Ith predicted moisture content (% d.b.)
RME	Relative mean error (%)
N	Number of data points
Qst	Isosteric heat of sorption (kJ/mol)
r	Correlation coefficient
R	Universal gas constant (8.314 J/mol·K)
SEM	Standard error of moisture
T	Absolute temperature (K)
r.h	Relative humidity

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1. Introduction

The *Pistacia atlantica* is a powerful tree, which can be 20 m tall, with an individualized anatomy trunk and deciduous leaves [1]. The analysis of processes that have impact on medicinal and aromatic products stability is based mainly on sorption isotherms. They also provide information on the mechanisms of sorption and interactions of biological product with water. Because of a complex structure of these products it is a complicated task to formulate analytical relationships to predict sorption behavior of products. An addition or removal of water changes the composition and dimensions of products and may induce phase changes such as dissolution and crystallization of sugars. A change in water content as a function of water activity is determined by various factors. Each of them can be predominant in a specified range of the sorption isotherm.

Literature does not provide any number of mathematical equations of sorption isotherms of occidental Algeria pistachio leaves. In practice, a temperature dependent equation is more convenient as only one set of parameters is needed in describing isotherms at different temperatures [2]. The knowledge of sorption isotherms at different temperatures enables an evaluation of the heat of sorption, which determines the interaction between an adsorbent and absorbate. Water availability in the reactions of product degradation depends both on water content and on the properties of diffusion surface on the thermodynamic function of sorbed water. The aim of this study was to investigate the effect of temperature on sorption properties of pistachio leaves (*Pistacia atlantica*). The scope of the investigation covered fitting of the experimental data to sorption isotherm equations to which references were made most often in the literature and which included a temperature factor and to determine the net isosteric heat of water sorption from the experimental data.

2. Materials and Methods

2.1 Experimental Procedure

The Atlantics pistachio leaves (*Pistacia atlantica*) used for the experiments have been grown in Bechar region of Algeria (Fig. 1). Harvest was between June and July 2006.

Fresh leaves of *Pistacia atlantica* were used in desorption experiments. Samples used in adsorption isotherms were dried in an oven regulated at a temperature of 105 °C, until they reached maximum dehydration.

In the present work, the static gravimetric method was used. This method is based on the usage of saturated salt solutions to maintain a fixed r.h. and has the advantage of presenting a more restricted domain of moisture content variation [3]. The mass transfers between the product and ambient air are saturated by natural diffusion of water vapor. The atmosphere surrounding the product has fixed air moisture content for every temperature applied to the salt solution [4].

Six salts were chosen (KOH, $MgCl_2$, K_2CO_3, $NaNO_3$, KCl and $BaCl_2$) to provide a range of 5%-90% relative humidity [5]. The experimental apparatus consisted of six glass jars of 1-l each with an insulated lid. Every glass jar was quarter filled with a saturated salt solution. Duplicate samples each of 0.1 g (± 0.0001 g) for desorption and 0.05 g (± 0.0001 g) for adsorption was weighed into glass jars. The six samples were weighed every day. Equilibrium moisture was recognized when three consecutive weight measurements showed a difference of less than 0.001 g. The equilibrium moisture content (EMC) of each sample was determined by a drying oven whose temperature was fixed at 105 °C for 24 h. The hygroscopic equilibrium of *Pistacia atlantica* leaves was reached in eight days for desorption and seven days for adsorption.

EMC is given by:

$$Xe_q = M_h - M_s/M_s \quad (1)$$

The temperature of the thermostatic bath was changed, and the same experiment was repeated for both adsorption and desorption processes at 40, 50 and 60 °C. Desorption and adsorption isotherm of *Pistacia atlantica* describing the hysteresis phenomenon for these three temperatures is given in Figs. 2-4.

2.2 Sorption Isotherm Equations

The description of the relationship between EMC, Aw and temperature was verified according to the following nine models selected for fitting the experimental data sorption isotherms of *Pistacia atlantica*: GAB, BET, Henderson, Oswin, Caurie, Peleg, Smith, White & Eiring and Langmuir. The selected equations are given in Table 1.

The quality of the model is determined by the values:

of the coefficient of correlation (r) given by the software Curve Expert 3.2;

of the relative mean error (RME) given by the relation:

$$RME = \frac{100}{N}\sum_{i=1}^{N}\left|\frac{Xeq_{i,\,\exp} - Xeq_{i,\,pre}}{Xeq_{i,\,\exp}}\right| \quad (2)$$

Fig. 1 Atlantics pistachio (*Pistacia atlantica*) of Algeria occidental.

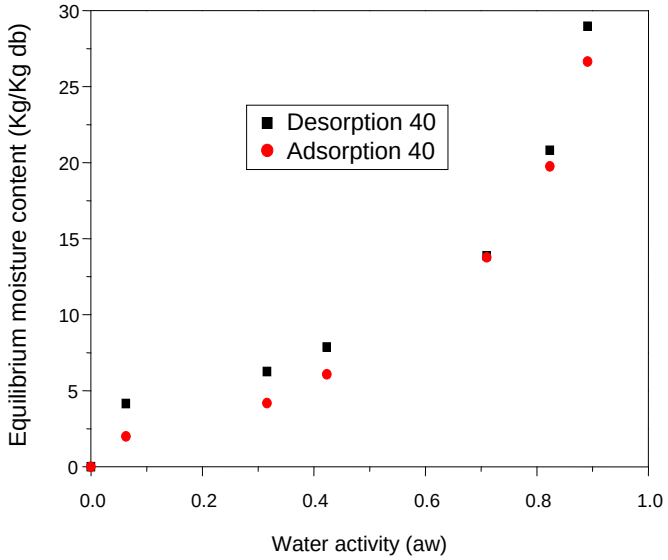

Fig. 2 Desorption and adsorption isotherms of Algeria occidental Atlantics pistachio (*Pistacia atlantica*) describing the hysteresis phenomenon for T = 40 °C.

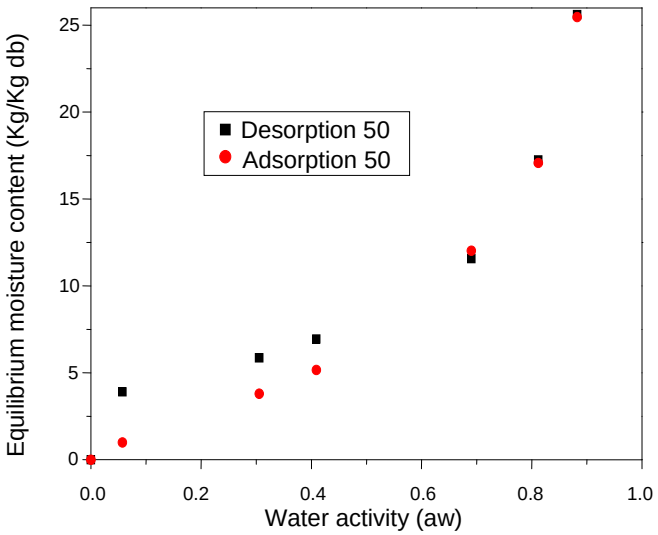

Fig. 3 Desorption and adsorption isotherms of Algeria occidental Atlantics pistachio (*Pistacia atlantica*) describing the hysteresis phenomenon for T = 50 °C.

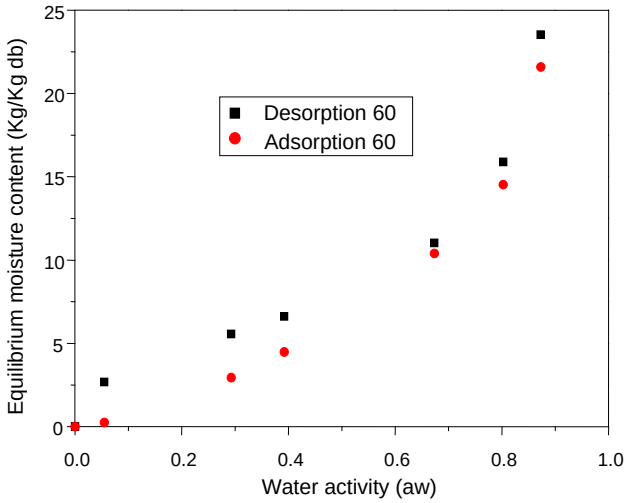

Fig. 4 Desorption and adsorption isotherms of Algeria occidental pistachio Atlantics (*Pistacia atlantica*) describing the hysteresis phenomenon for T = 60 °C.

Table 1 Models for fitting the sorption isotherm of *Pistacia atlantica*.

Auteurs	Equation du modèle	References
Henderson	$Xeq = -(\ln(1 - (e.r.h))/A(T + B))^{1/C}$	Thompson et al. [6]
Oswin	$Xeq = (a + (BT)) ((e.r.h)/(1 - (e.r.h))^{C}$	Oswin [7]
GAB	$Xeq = ABC (e.r.h)/(1 - (e.r.h)) (1 - B (e.r.h) + BC(e.r.h))$	Berg [8]
BET	$Xeq = x/(a + b \times x - (a + b) \times x \times x)$	Iglesias and Chirifie [9]; Chirifie and Iglesias [10]
Caurie	$Xeq = (e.r.h)/((a + b(e.r.h) - (a + b)(e.r.h)(e.r.h))$	Castillo et al. [11]
Peleg	$Xeq = Exp((a + b(e.r.h))$	Peleg [12]
Smith	$Xeq = A(e.r.h)^{k1} + B(e.r.h)^{k2} A - B[\ln(1 - (e.r.h))]$	Smith [13]
White & Eiring	$Xeq = 1/(A + B(e.r.h))$	Castillo et al. [11]
Langmuir	$Xeq = 1/(A + B(e.r.h)^{\hat{C}-1})$	Langmuir [14]

The standard error of the moisture content of the plant (*SEM*) expressed by Eq. (3):

$$SEM = \sqrt{\frac{\sum_{i=1}^{N} (Xeq_{i,\,exp} - Xeq_{i,\,pre})^2}{d_f}} \quad (3)$$

2.3 Determination of the Isosteric Heat of Sorption

The isosteric heat of sorption (Q_{st}) can be expressed by the equation of Clausius Clapeyron [15-17], as follows:

$$Q_{st} = -R\left[\frac{\partial(\ln Aw)}{\partial(\frac{1}{T})}\right] \quad (4)$$

The integration of the Eq. (4), supposing that the isosteric heat of sorption is independent from the temperature, gives the following equation:

$$\ln(Aw) = -(\frac{Qst}{R}).\frac{1}{T} + K \quad (5)$$

The value of Q_{st} was calculated from the slope of the plot between the values of ln(Aw) and 1/T at constant moisture content, the nonlinear optimization method of Marquardt-Levenberg [16, 17], using the program of calculation Curve Expert 3.2, and Origin 6.1 is used to look for the best equation of the isosteric heat of sorption (Q_{st}) of Atlantics pistachio leaves of *Pistacia atlantica*.

3. Results

3.1 Biochemical Analysis

The results of biochemical analysis of dried leaves of *Pistacia atlantica* are shown in Table 2.

3.2 Sorption Isotherm of Pistacia atlantica

The experimental desorption and adsorption isotherms obtained at 40, 50 and 60 °C are shown in Figs. 2-4.

3.3 Modeling of Sorption Isotherms

The results of the measures of the equilibrium moisture content (X_{eq}) of adsorption and desorption are shown in (Tables 3 and 4). Adsorption and desorption isotherm of *Pistacia atlantica* described by GAB and PELEG equation are shown in (Figs. 5-8).

3.4 Isosteric Heats of Sorption

The isosteric heat of adsorption and desorption of *Pistacia atlantica* leaves is determined by using the GAB model for various moisture contents is given in Figs. 9 and 10.

Table 2 Results of biochemical analysis.

	Global chemical composition of dried *Pistacia atlantica* leaves
Lipids (%)	2.25
Protéin (%)	4.35
Carbohydrates (%)	25.77
Moisture (%)	33

Table 3 The equilibrium moisture content of adsorption of the pistachio leaves (*Pistacia atlantica*).

Adsorption 40 °C	*Aw*	0.0626	0.3159	0.423	0.71	0.8232	0.891
	X_{eq}	2.004	4.1916	6.0852	13.7725	19.7605	26.6533
Adsorption 50 °C	*Aw*	0.0572	0.3054	0.4091	0.6904	0.812	0.8823
	X_{eq}	0.994	3.7924	5.1587	12.022	17.0732	25.462
Adsorption 60 °C	*Aw*	0.0549	0.2926	0.3921	0.6735	0.8025	0.8728
	X_{eq}	0.2469	2.9484	4.4776	10.396	14.532	21.5881

Table 4 The equilibrium moisture content of desorption of the pistachio leaves (*Pistacia atlantica*).

Desorption 40 °C	*Aw*	0.0626	0.3159	0.423	0.71	0.8232	0.891
	X_{eq}	4.1513	6.2615	7.8755	13.8728	20.8178	28.9720
Desorption 50 °C	*Aw*	0.0572	0.3054	0.4091	0.6904	0.812	0.8823
	X_{eq}	3.9007	5.8621	6.9444	11.5694	17.2414	25.5973
Desorption 60 °C	*Aw*	0.0549	0.2926	0.3921	0.6735	0.8025	0.8728
	X_{eq}	2.6769	5.5556	6.6176	11.0307	15.8879	23.5294

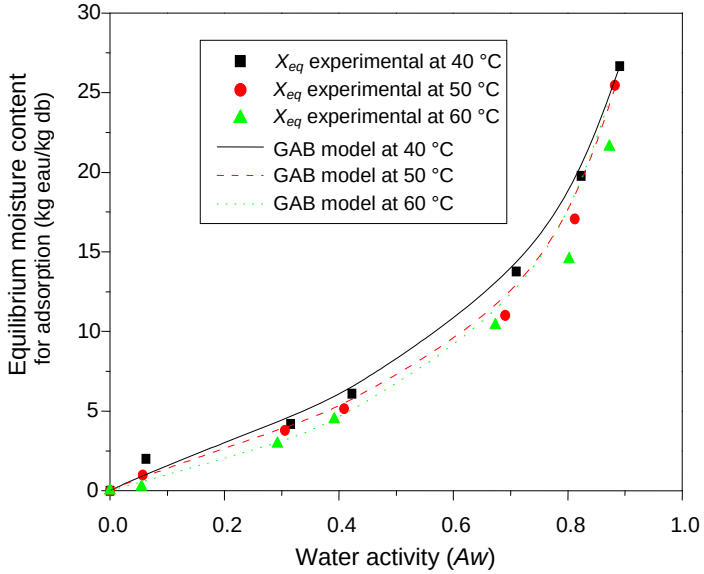

Fig. 5 Adsorption isotherm of *Pistacia atlantica* described by GAB equation.

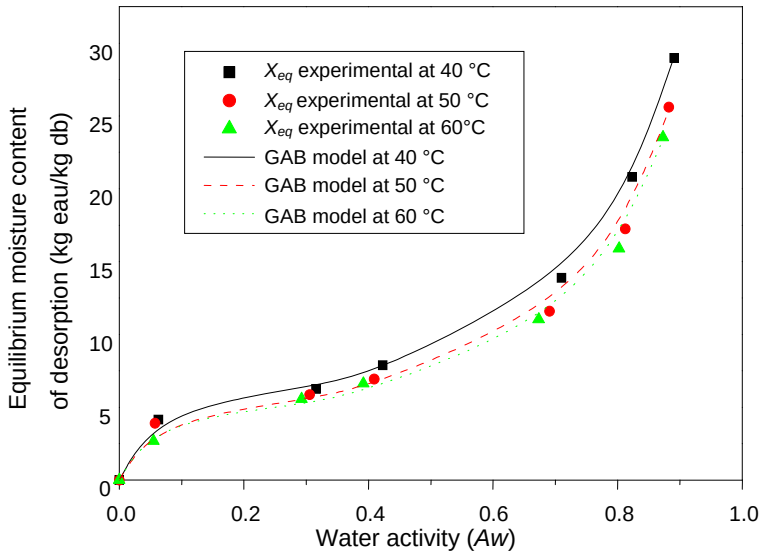

Fig. 6 Desorption isotherm of *Pistacia atlantica* described by GAB equation.

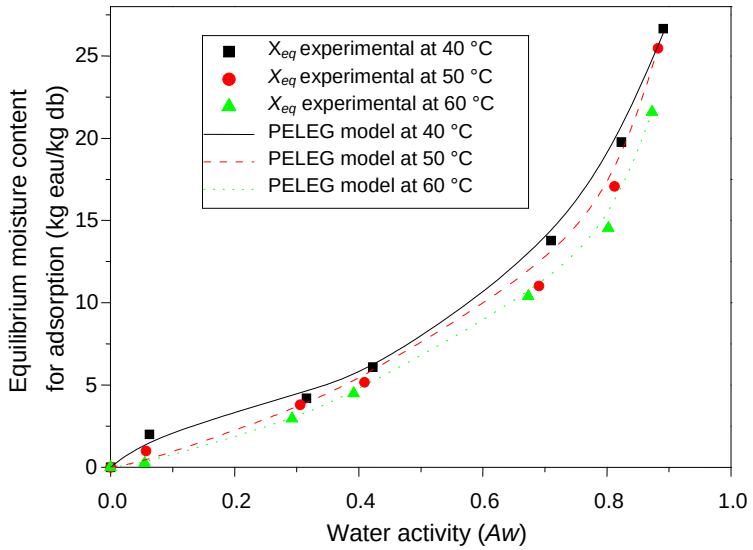

Fig. 7 Adsorption isotherm of *Pistacia atlantica* described by PELEG equation.

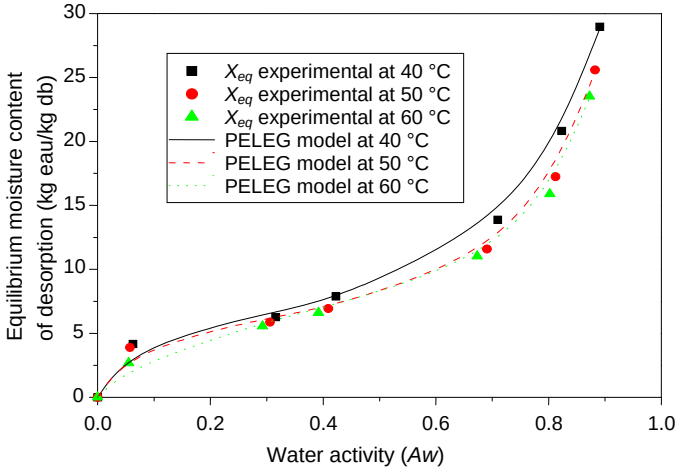

Fig. 8 Desorption isotherm of *Pistacia atlantica* described by PELEG equation.

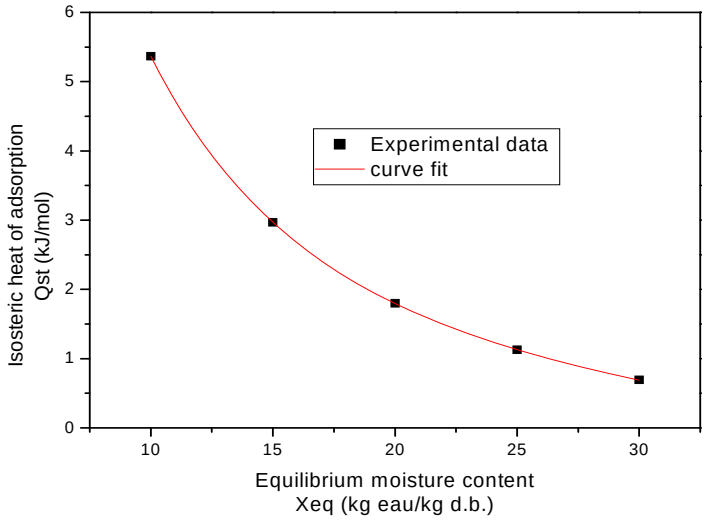

Fig. 9 Isosteric heat of adsorption of the *Pistacia atlantica* (GAB).

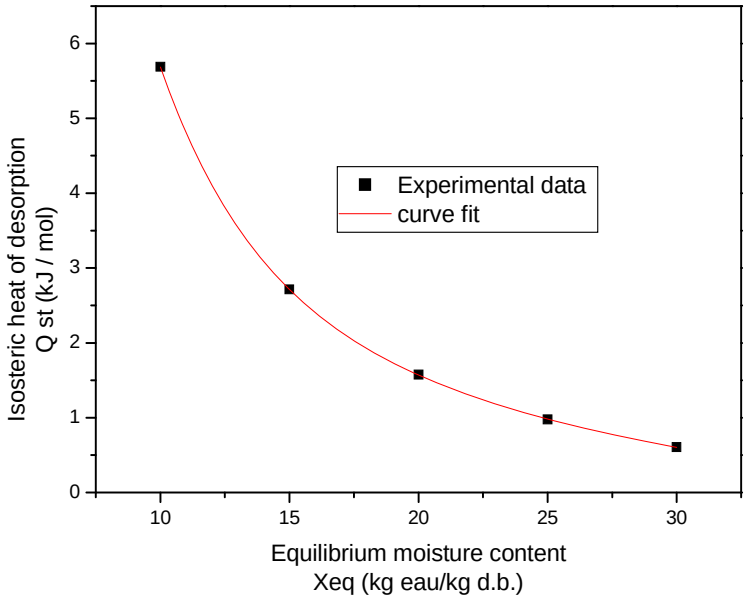

Fig. 10 Isosteric heat of desorption of the *Pistacia atlantic*a (GAB).

4. Discussion

4.1 Biochemical Analysis

The leaves have a pistachio lipid fraction equal to 2.25%; proteins are the second macronutriment that predominates in these sheets: 4.35%. Accordingly carbohydrates content was about 25.77%. Fresh *Pistacia atlantica* leaves are feeble moisture products: the moisture content of the leaves was about 33%. According to this result, the pistachio leaves could be considered as a rich natural source of valuable nutriments (carbohydrates, proteins and lipids).

4.2 Sorption Isotherm of Pistacia atlantica

Sorption isotherms of *Pistacia atlantica* have the sigmoid form and behave like a type II on BET classification. These curves are typical of plant products as reported in literature by Jamali et al. [16-25].

As seen in the figures presented the experimental desorption and adsorption isotherms obtained at 40, 50 and 60 °C; the equilibrium moisture content increased

with water activity at constant temperature. There is also a decrease in the equilibrium moisture content with increasing temperature, at a constant water activity; this can be explained by the change in the excess enthalpy of water binding, dissociation of water, or increase in solubility of solute in water as temperature increases [26-28]. The figures also show the super imposition between adsorption and desorption isotherms does not match, this mismatch between adsorption and desorption isotherms is called "pseudo-hysteresis" [29]. This phenomenon can lead to the wrong conclusion that a part of solute cannot be desorbed at all, whereas it could be a matter of time [30-34]. On the other hand, the saturation capacity of the solid during adsorption can be underestimated if equilibrium is not reached [35].

4.3 Modeling of Sorption Isotherms

The results of the measures of the equilibrium moisture content (X_{eq}) of adsorption and desorption confirm that for an activity given of water, the equilibrium content (X_{eq}) decreases when the temperature increases.

The same method of Marquardt-Levenberg is used to calculate the coefficients as well as the static parameters of nine models chosen to describe sorption curves of *Pistacia atlantica* (Table 5); that indicate that all the models are acceptable for predicting the equilibrium moisture content. However, the GAB and Peleg models gave the best fitting of adsorption and desorption isotherms for the three temperatures, with lowest standard error and the highest coefficient of determination (r). The model of GAB (semi-empiric) uses five parameters and takes into account the effect of the temperature. The GAB and Peleg models describe best the isotherms of sorption of Atlantics pistachio (*Pistacia atlantica*) for the strong activities of the water (0.05-0.9). The GAB and Peleg equations were found to be satisfactory for many other plant species like Olive leaves by Boudhrioua [35]. On the other hand, the same results are obtained with BET's model. It was an adequate model for describing the sorption isotherms of *Pistacia atlantica* but the BET's model was defined only for the domain of the feeble water activities (*Aw* less than 0.5).

Figs. 5-8 give the experimental values of the equilibrium moisture contents of sorption of the *Pistacia atlantica* at three working temperatures (40, 50 and 60 °C) and for a water activity vary of 5%-90% as well as the curves calculated by GAB and Peleg equations. For a given water activity, the equilibrium moisture content decreases when the temperature increases. This result is in conformity with many research works on the other plants and products [36, 37].

4.4 Isosteric Heats of Sorption

The isosteric heat of adsorption and desorption of *Pistacia atlantica* leaves is determined by using the GAB model for various moisture contents (Figs. 9 and 10); the same result was obtained by Blahovec [38]. The isosteric heat of sorption decreases when the moisture content increases [39]. The slope devolvement is strong for the feeble moisture contents.

Tsami [40] explains that the high value of the sorption heat at feeble moisture content was done to the existence of strongly active polar places on the product surface. Water molecules form a monomolecular layer. We notice that the isosteric heat of desorption is bigger than that of the adsorption. This indicates that the energy required in the process of desorption is superior to that necessities in the process of adsorption as indicated. The isosteric heat of desorption and of adsorption of the *Pistacia atlantica* leaves decreases with an increase of the equilibrium moisture content (Figs. 9 and 10). It is expressed as exponential function of second order of the equilibrium moisture content.

The isosteric heat of sorption of water of the *Pistacia atlantica* can be expressed by an exponential function of second order of the moisture content. This relation can be employed to calculate the sorption heat of pistachio leaves for various humidity [40].

Table 5 Performance of the models selected for the sorption isotherms of the *Pistacia atlantica*.

Modèle	Constants	Desorption			Adsorption		
		40 °C	50 °C	60 °C	40 °C	50 °C	60 °C
BET	A	0.0024870029	0.0018696018	0.0093691564	0.019373812	0.0519	0.14583637
	B	0.21603485	0.2373944	0.21445475	0.23510838	0.1514	-0.15297496
	C						
	r	0.9996	0.9999	0.9999	0.9927	0.9998	0.9989
	EMR (%)	1.8484	0.0504	0.3636	3.5324	1.0653	10.8069
	EST	0.1434	0.0036	0.0222	0.4862	0.0761	0.1691
Caurie	A	0.3871	0.3701	0.3453	-0.5058	-0.2184	0.2271
	B	3.1469	3.2588	3.36969	4.1305	3.8539	3.437
	C						
	r	0.979	0.9967	0.9769	0.9966	0.9878	0.9907
	EMR (%)	20.4736	20.1832	21.0790	42.9775	12.5622	13.7790
	EST	2.2535	2.2734	2.7246	1.1925	1.4901	1.7050
GAB	A	0.94795037	0.95643882	0.96295543	0.90207904	0.92923527	0.9429
	B	3.3680431e+09	357.71755	1.4542088e+11	3.6927312	3.0260776	2.2617
	C	4.5206376	3.9647212	3.7297884	5.580784	4.3390056	4.26e+00
	r	0.9994	0.9993	0.9967	0.9989	0.9981	0.9981
	EMR (%)	4.1488	3.0952	11.8042	10.5090	3.6070	27.5917
	EST	0.4641	0.4495	0.9286	0.6341	0.4437	2.2163
Oswin	A	0.1311	-20.3108	-25.2412	-16.7482	-21.294	-25.6665
	B	-34.623	0.5737	0.5627	0.5562	0.5541	0.5555
	C	0.1311	0.5719	0.6113	0.7164	0.6811	0.6368
	r	0.9849	0.996	0.99	0.9994	0.9998	0.9982
	EMR (%)	12.2447	13.3733	15.1804	38.9206	2.3173	9.2608
	EST	1.1457	1.6239	1.9093	0.4425	0.2364	0.7979
Langmuir	A	-1.5163	-2.4235	-1.8381	-0.3622	-0.8419	-0.9825
	B	1.5362	2.4441	1.8515	0.3781	0.8563	0.9979
	C	0.8869	0.9377	0.9122	0.4489	0.7679	0.8336
	r	0.9939	0.9913	0.9891	0.9995	0.9993	0.9976
	EMR (%)	15.1416	15.7784	16.6417	34.7165	7.2085	7.5208
	EST	1.3905	1.6547	1.9152	0.3820	0.4733	0.9180
Henderson	A	0.1311	0.139	0.1085	0.1339	0.1215	0.1109
	B	-34.623	-25.6895	53.378	-34.5065	-43.8027	-53.2346
	C	0.1311	0.139	0.1085	0.1339	0.1215	0.1109
	r	0.9849	1.7674	0.977	0.9888	0.9909	0.9914
	EMR (%)	23.5242	25.0976	24.6698	42.1963	13.8500	15.6762
	EST	2.1996	2.6016	2.9721	1.9612	1.7758	1.7722
Smith	A	0.7798	0.7243	0.7967	-0.9409	-0.2676	0.128
	B	10.3261	11.5303	12.2804	10.8324	-8.357	11.9307
	C						
	r	0.9869	0.9895	0.979	0.9582	0.9862	0.9926
	EMR (%)	16.1893	15.9275	16.7808	42.8083	19.2808	11.6041
	EST	1.9997	2.1935	2.5986	1.5960	1.7049	1.9976
White & Eiring	A	0.2988	0.2854	0.2901	0.3641	0.3453	0.3127
	B	-0.2919	0.2825	-0.2933	-0.3642	-0.3476	-0.3162
	C						
	r	0.9855	2.2067	0.9798	0.9796	0.9854	0.9766
	EMR (%)	25.4966	24.7092	29.9599	51.2620	45.0421	42.4795
	EST	1.6895	1.8030	2.1522	1.6220	1.7749	1.8362

(Table 5 continued)

Modèle	Constants	Desorption			Adsorption		
		40 °C	50 °C	60 °C	40 °C	50 °C	60 °C
Peleg	A	39.26268	9.2167632	42.731167	66.8005	19.940922	18.964871
	B	6.1274888	0.32507713	8.863349	13.302	1.4355237	1.5286467
	C	9.8460613	40.697793	11.447932	18.3015	65.887664	121.39816
	D	0.3308571	7.1775236	0.54781607	1.1281	16.079778	21.882456
	r	0.9995	0.999	0.9991	0.9981	0.9994	0.9999
	EMR (%)	3.4173	3.7641	4.8330	16.8805	14.3978	2.0292
	EST	0.5318	0.6517	0.5753	2.0593	0.8224	0.0559

Adsorption:

$$Q_{st} = -1.30373 + 20.5779 \exp(-X_{eq}/5.17645) + 5.09939 \exp(-X_{eq}/30.88933) \quad (6)$$

($r = 1$)

Desorption:

$$Q_{st} = -0.57534 + 45.66915 \exp(-Xeq/3.67826) + 5.42492 \exp(-X_{eq}/19.51286) \quad (7)$$

($r = 0.99999$)

5. Conclusions

The pistachio leaves could be considered as a rich natural source of valuable nutriments. The sorption isotherms of *Pistacia atlantica* were experimentally determined for three temperatures (40, 50 and 60 °C). The authors conclude that the GAB and Peleg models coincide with the experimental points in the field of water activities going from 5% to 90%. These results correspond to a preliminary stage for the study of the convective kinetics of the drying.

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Comparison of Sensory Characteristics of Green Tea Produced in Thai Nguyen and Phu Tho Province, Vietnam

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Abstract: Green tea is a popular product with a high consumption in Vietnam. Moreover, green tea produced in Tan Cuong commune, Thai Nguyen province, has long been recognized for its superior quality to those coming from other regions of the country. This study aims at comparing the tea from Thai Nguyen and Phu Tho and finding out if the difference can be identified through sensory analysis. Two products picked from Tan Cuong, Thai Nguyen province, and two others from Phu Ho district, Phu Tho province were evaluated by a trained panel of 12 judges (11 women and 1 man). The sensory evaluation of the dry tea (11 descriptors) was carried out separately from the brewed tea (21 descriptors) and brewed leaf (five descriptors) using an unstructured intensity scale (10 cm). Statistic analyses have shown differences between regions in all three groups of attributes: dry leaf, liquor and brewed leaf.

Key words: Green tea, sensory analysis.

1. Introduction

Green tea is one of the most important sectors in Vietnam's beverage market, both in production and in consumption. The green tea production of Vietnam in 2010 reached 50,000 tonnes (FAO), only behind of China and Japan. Half of the green tea produced in Vietnam is destined to be consumed nationally (FAO); and, 89% of tea produced in Thai Nguyen, the main region in our research, is consumed inside Vietnam (statistics of province Thai Nguyen, 2012).

Green tea has been cultivated and processed in Thai Nguyen province for hundreds of years and products of this region are recognized for their superior quality [1-3]. Tan Cuong, a commune in Thai Nguyen province, was granted the Geographical Indication in 2008 for its differentiated quality. However, in reality, the products of Thai Nguyen, especially of Tan Cuong, have been counterfeited within the market, which reduces the confidence of the consumers in the products. Research to distinguish the Thai Nguyen products from other products is deemed necessary to protect the pre-existing image of Thai Nguyen products. Other sensory researchers of Thai Nguyen green tea in Vietnam had recognized variations in quality; however, the research is based only on the general notes given by the experts on tea in Vietnam, who did not describe in detail the characteristics of the products. Our method of research will be a sensory analysis of the taste profile of different teas, based on the opinion of judges evaluating a list of descriptor attributes.

These days the development of productivity and quality in the Thai Nguyen tea region is derived from the local tea variety (Trung Du) and acclimatized tea

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varieties, which originate in other regions. They include LDP1, a product of the Northern Mountainous Agriculture and Forestry Science Institute (Nomafsi), which is a hybrid of the plants Dai Bach Tra (China) and PH1 (selected from *C. Assamica* India population), and Kim Tuyen, a tea variety which was imported from Taiwan. Phu Tho is a mountainous region in the North of Vietnam at the same elevation as Thai Nguyen. The culture of tea in Phu Tho is also based on LDP1 and Kim Tuyen. Therefore, these areas are very compatible because of their similar characteristics of attitude, topography and climate.

The goal of this study is to discover if the difference between products of different regions can be identified through sensory analysis of the taste profile of tea varieties Kim Tuyen and LDP1. However, in the framework of this article, we are only presenting the results of the Kim Tuyen variety in detail, as it is the focus of this study.

2. Material and Method

2.1 Samples

Four Kim Tuyen dried green tea products were selected for analysis. Two of them were from Tan Cuong marked as TKH1 (A) and TKH2 (B). The two others are from Phu Ho, marked as PKH1 (A) and PKH2 (B). These tea products are considered some of the best in the aforementioned regions according to the evaluations of the local people and the green tea competition in the region each year. For all products, the raw materials used were the fresh teas picked in the following way: one bud and two leaves, both harvested at the same time (around October 2012). After the harvest, each tea underwent the same processing (Fig. 1).

After processing in the household in the village, the product is called "che moc cau" which will be used for the experiments. Table 1 below presents a list of the products. The products are packaged in a 200 g bag comprised of two sheets, plastic and aluminum. After manipulation, the products are transferred to the laboratory and stored. It is stored under cover in a temperature-controlled room, where the temperature does not exceed 28 °C.

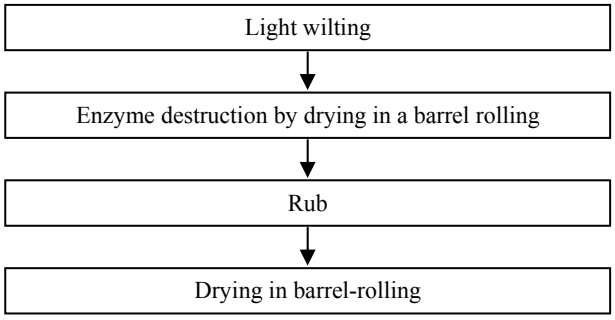

Fig. 1 Flowchart illustrating how green tea is processed following the harvest.

Table 1 List of the products.

Product's name-mark	Product code	Product code simplified
Thang Huong, Tan Cuong	TKH1	A
Tien Yen, Tan Cuong	TKH2	B
Nomafsi, Phu Ho	PKH1	C
Ba Lu, Phu Ho	PKH2	D

2.2 Sensory Analysis

Thirty-eight people participated in the panel recruitment. They carried out two basic sensory tests focusing on smell, taste and color. The 12 people with the best results in their distinctions between smells, tastes and colors concerning the green tea were chosen to be judges for further training. During the training process, panelists were introduced and instructed on the products and references for each attribute. The judges have tested the products using various additives and concentrations of these additives and then discussed the results. After 20 training hours, the panelists began evaluating the intensity of the attributes of dried tea, liquor and infused leaf.

The tests were carried out using a method involving an unstructured graphical scale. The scores of individual evaluations of all tea quality parameters were marked on a 10 cm line scale, where the left side designated the low notes for the attribute, and the right side designated the high notes. For each attribute, the judges evaluated all four products using the same scale. The products were identified only by a random 3-digit

code. The judges did not know which product the codes represented. The closer the products are on the scale, the more similar the products are on that attribute.

The tea brewing method used in this study is in compliance with standard TCVN 5086-90 (or ISO 3103-1980). An amount of 2.8 g dried tea was put into a 140 mL brewing cup. Boiled water from a rapid electric kettle was poured onto the dried tea (water temperature in the infuser is about 93 °C) and timing was started. After 6 min, the tea was poured into a tasting cup. The tea temperature in the tasting cup is about 70 °C. The order of the presentation of the products at each stage of testing is altered for each judge. This ensures the reliability with respect to the balance of the products.

In reference to standards TCVN 3218-1993, 10 TCN 258-96, ISO 6078-1982 and the literature review on green tea sensory attributes [4, 5], a list of lexicons was approved by an internally trained panel and the group of judges selected for the final evaluation. Table 2 presents the selected sensory attributes which denote the products description.

The vertical lines marked by a panelist on each scale of each attribute were converted to numerical values by measuring the distance in centimeters between the left end of the scale and the marker of the product. Analysis of variance (ANOVA) using the Tukey test method and principal component analysis (PCA) were performed by Statistica 8 and SPAD 5.5 software, respectively.

3. Results and Discussion

3.1 Dry Leaf

Average intensities of each attribute are presented in Table 3. The small letters on the top right of the average values display the results of the Tukey test (α = 0.05). Products that were marked by the same letter belong to the same group. The last column expresses average values in ascending order for each attribute. In this comumn, if the products Tan Cuong (A, B) and the products Phu Tho (C, D) are isolated, the order is marked with an asterisk (*).

The results show a significant difference between the two regions on 10 of the 11 attributes: brown, black, green, silver, downy, leafy, crepy, wiry and blister. For example, on the green attribute, Phu Tho tea products have an average value of 1.68-2.23 while Tan Cuong tea products have a bigger average value of 5.43-5.98. The different ordering of average values

Table 2 Attributes for green tea description.

No.	Categories	Aspects	Vietnamese wording	English
1	Dry leaf	Appearance	Xanh	Green
2			Đen	Black
3			Vàng	Brown
4			Bạc	Silver
5		Color	Xoăn	Crepy
6			Dài cánh	Length
7			Chắc cánh	Wiry
8			Bồm	Leafy
9			Phồng rộp	Blister
10			Cẵng	Fibrous
11			Tuyết	Downy
12	Brewed tea	Appearance	Độ trong	Clarity
13			Độ sáng	Bright
14			Độ sánh	Body
15			Xanh	Green
16			Vàng	Yellow
17		Smell	Hoa nhài	Jasmine-like
18			Hoa hồng	Rosy-like
19			Mùi cỏ xanh	Green herb-like
20			Cốm	Young green rice
21			Mùi chè non	Flavour
22			Mùi chè mới	New (><mouldy)
23			Hăng xanh	Sour
24			Ôi ngốt	Fermented
25			Khói	Smoky
26			Cao lửa	Over-fired
27		Taste	Chát	Astringent
28			Đắng	Bitter
29			Ngọt	Sweet
30			Umami	Umami
31			Hậu vị	Recurring sweetness
32			Giảm hậu vị	Less sweetness after tasting
33	Leaf brewed	Color	Màu xanh lá	Green
34			Màu đồng	Coppery
35			Độ sáng	Bright
36		Appearance	Độ lớn	Grade
37			Độ mềm	Soft

Table 3 Average value of dry tea attributes.

	Attribute	TKH1 (A)	TKH2 (B)	PKH1 (C)	PKH2 (D)	*P*-value	Ascending order of evaluation
1	Green	6.0[b]	5.4[b]	2.2[a]	1.7[a]	0.000	DCBA*
2	Black	6.9[b]	6.0[b]	2.4[a]	2.6[a]	0.000	CDBA*
3	Brown	2.4[a]	3.5[b]	7.5[c]	7.9[c]	0.000	ABCD*
4	Silver	6.5[b]	6.2[b]	3.2[a]	2.0[a]	0.000	DCBA*
5	Downy	8.4[c]	5.5[b]	3.0[a]	1.6[a]	0.000	DCBA*
6	Fibrous	1.5[a]	1.9[a]	8.2[b]	8.5[b]	0.000	ABCD*
7	Leafy	2.0[a]	3.0[b]	8.1[c]	8.3[c]	0.000	ABCD*
8	Crepy	6.9[c]	4.7[b]	2.7[a]	2.0[a]	0.000	DCBA*
9	Wiry	6.8[c]	5.5[b]	1.7[a]	1.7[a]	0.000	DCBA*
10	Blister	1.8[a]	3.0[a]	6.1[b]	4.6[b]	0.000	ABDC*
11	Length	6.6[b]	2.9[a]	8.5[c]	6.0[b]	0.000	BDAC

[abc] the same letter belongs to the same group; * Tan Cuong and Phu Tho products are isolated.

for the "Length" attribute can be explained by the lack of sufficient consideration given to length as an attribute during the research. In addition, the length of the product is susceptible to change in transport.

Therefore, with the same picking criteria (one bud and two leaves), Tan Cuong brought back a product which is more silver, greener, blacker, wirier, more crepy, more downy, but less brown, less leafy, less fibrous, less blister. According to green tea standards (standard green tea is black green, less leafy, less fibrous and more downy (TCVN 3218:1993)), the Tan Cuong tea has better notes, because of the differences in geography, climate, cultivation practices and processing method. Research has shown that good cultivation practices, such as the use of fertilizers, play an important role in tea production in the Tan Cuong tea regions [6-8].

Fig. 2 shows the result of PCA on product description. Principal component 1 explained 61.19% of the total variance and principal component 2 explained 9.6% of the total variance.

Factor 1 is represented with attributes such as green, silver, downy, crepy, black, brown, fibrous and leafy. Among those, yellow, fibrous and leafy are in the opposite direction of the others. These are attributes that distinguish products of Tan Cuong and Phu Ho. Four products are projected on four different quarters. Products of Tan Cuong differ from those of Phu Ho, the difference between regions can be noted as green, silver, downy, crepy, black, brown, fibrous, leafy.

3.2 Brewed Tea

3.2.1 Color and Appearance

Having a similar structure to Table 3, Table 4 shows the results of Tukey test analysis on attributes of color and appearance of brewed tea.

The results do not show the differences of tea between Tan Cuong and Phu Tho, even though the differences between products exist.

3.2.2 Arome

By looking at the ANOVA in Table 5 which shows the results of Tukey test on attributes of aroma of brewed tea, we see the regional differences for six attributes: sour, fermented, new, flavour, rosy-like and young green rice.

The most differences can be seen between rose and young green rice scent. Tan Cuong tea's rose scent is weaker and young green rice's scent is stronger. This fact highlights that Tan Cuong teas are processed at higher temperatures compared to Phu Ho's. This processing at high temperatures influences young green rice's scent, but also reduces the flower-scent of the tea (for Kim Tuyen tea, the most specific flower scent is rosy-like).

With the attributes sour, fermented, new and flavour, products of the two regions are distributed on two sides, though mixed products are not discriminated in term of statistics. For example, new tea aroma,

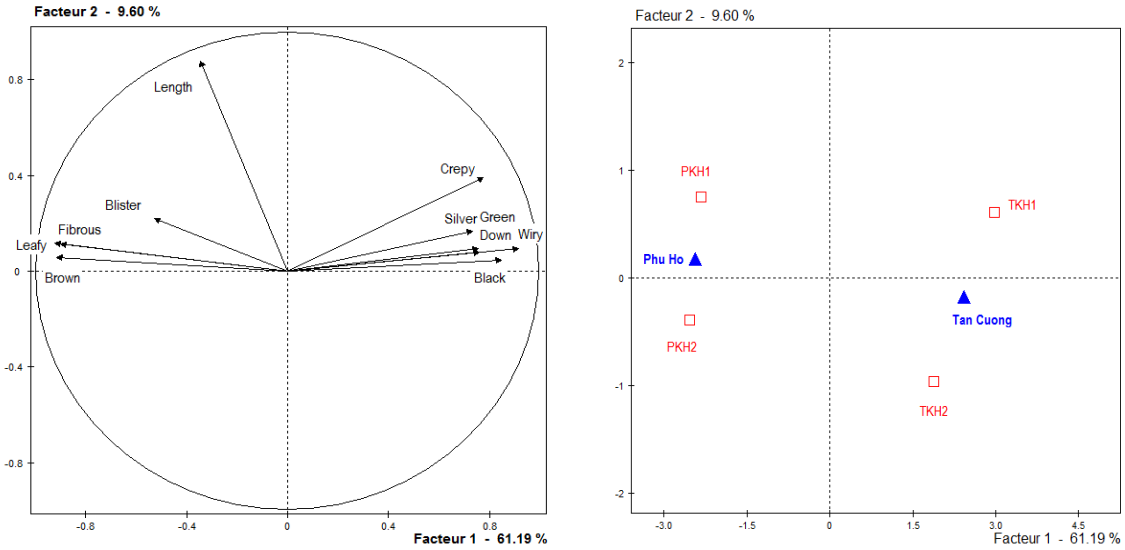

Fig. 2 PCA analysis on dry tea. Left: correlation of the attributes with the first two components of the PCAs; right: projection of the products onto the first two PCs.

Table 4 Average values of color and appearance attributes of brewed tea.

No.	Attribute	TKH1 (A)	TKH2 (B)	PKH1 (C)	PKH2 (D)	*P*-value	Order of evaluation
1	Clarity	5.6^{a}	6.1^{ab}	6.9^{b}	5.9^{ab}	0.039	ADBC
2	Bright	6.4^{b}	6.1^{b}	7.1^{b}	3.8^{a}	0.000	DBAC
3	Body	4.3^{a}	5.4^{ab}	4.4^{a}	6.1^{b}	0.001	ACBD
4	Green	3.6^{a}	6.0^{c}	4.6^{b}	8.1^{d}	0.000	ACBD
5	Yellow	6.4^{c}	5.8^{b}	6.1^{c}	2.7^{a}	0.000	DBCA

^{abcd} the same letter belongs to the same group.

Table 5 Average values of aroma attributes of brewed tea.

No.	Attribute	TKH1 (A)	TKH2 (B)	PKH1 (C)	PKH2 (D)	*P*-value	Order of evaluation
1	Sour	3.8^{ab}	3.0^{a}	3.9^{ab}	4.4^{b}	0.066	BACD*
2	Over-fired	1.9^{a}	2.5^{a}	1.8^{a}	2.4^{a}	0.350	CADB
3	Smoky	3.0^{a}	2.2^{a}	3.2^{a}	2.9^{a}	0.302	BDAC
4	New (><mouldy)	5.4^{ab}	5.7^{b}	4.4^{ab}	4.1^{a}	0.006	DCAB*
5	Flavour	5.1^{bc}	5.6^{c}	4.0^{ab}	3.0^{a}	0.000	DCAB*
6	Jasmine-like	3.2^{a}	3.2^{a}	2.8^{a}	3.3^{a}	0.867	CABD
7	Rosy-like	1.7^{a}	1.8^{ab}	3.0^{b}	4.5^{c}	0.000	ABCD*
8	Young green rice	3.9^{b}	5.5^{c}	2.9^{ab}	2.2^{a}	0.000	DCAB*
9	Green herb-like	3.5^{a}	3.3^{b}	3.8^{a}	3.3^{a}	0.801	DBAC
10	Fermented	2.0^{ab}	1.3^{a}	2.7^{ab}	3.1^{b}	0.008	BACD*

^{abc} the same letter belongs to the same group.

PKH1 (4.41) belongs to both groups a (PKH1: 4.11) and b (TKH2: 5.71). More experiments with a larger number of samples are required to more strongly identify differences in regional tea varieties. However, based on the results of the experiments which took place, Tan Cuong has a higher value for new, flavour attributes and a lower value for sour and fermented attributes.

Thus, in conclusion, theses differences of aroma attributes such as sour, fermented, new, flavour, rosy-like and young green rice, are the distinguishing characteristic of the tea from Tan Cuong, which

expresses the difference in production technology, and in quality intrinsic to the tea leaf itself.

3.2.3 Taste

Among taste attributes, those most related by geography, origin and tea grade [9] are hardly differentiated at all by ANOVA analysis. Table 6 shows the results of Tukey test on taste attributes of brewed tea. The PCA results show that in comparison with Phu Tho products, Tan Cuong tea is characterized as astringent, bitter and umami (Fig. 3). The difficulties of regional differentiation in taste attributes could be caused by polyphenol compounds, which lead to bitterness and astringency (which are not favored by the consumer) in green tea. Indeed, this compound is difficult to evaluate on sensory analysis, especially with a large number of samples [10]. This situation can explain the confusion surrounding the origin of tea products on the market, as the regional related attributes are very hard to distinguish, especially to mass consumers.

In summary, we did PCA analysis for the following characteristic groups: color and appearance, aroma and taste. Though they are not distributed into two different regions, Tan Cuong products are similar to each other and tend to be near to Axis 2. After the analysis, we can see that Tan Cuong tea products are characterized by their flavour, new, green herb-like and young green rice aromas, and their astringent, bitter and umami tastes.

PKH2 products lie differentially and were characterized by Yellow, meanwhile other products TKH1, TKH2 and PKH1 are nearer to Green and Bright. Besides, appearance and color of green tea are affected mainly by technology process. PKH1 products have a defining characteristic of color and appearance and are closer to Tan Cuong products, which

Table 6 Average values of taste attributes of brewed tea.

No.	Attribute	TKH1 (A)	TKH2 (B)	PKH1 (C)	PKH2 (D)	*P*-value	Order of evaluation
1	Astringent	4.3[a]	3.3[a]	3.5[a]	3.5[a]	0.183	BCDA
2	Bitter	5.5[a]	4.7[a]	5.0[a]	5.1[a]	0.518	BCDA
3	Sweet	4.4[a]	4.5[b]	4.4[a]	4.2[a]	0.875	DACB
4	Umami	3.6[ab]	4.2[b]	3.0[a]	3.7[ab]	0.066	CADB
5	Recurring sweetness	4.3[a]	4.1[a]	4.3[a]	4.3[a]	0.978	BCDA
6	Less sweetness after tasting	3.8[a]	4.5[a]	3.4[a]	4.1[a]	0.244	CADB

[ab] the same letter belongs to the same group.

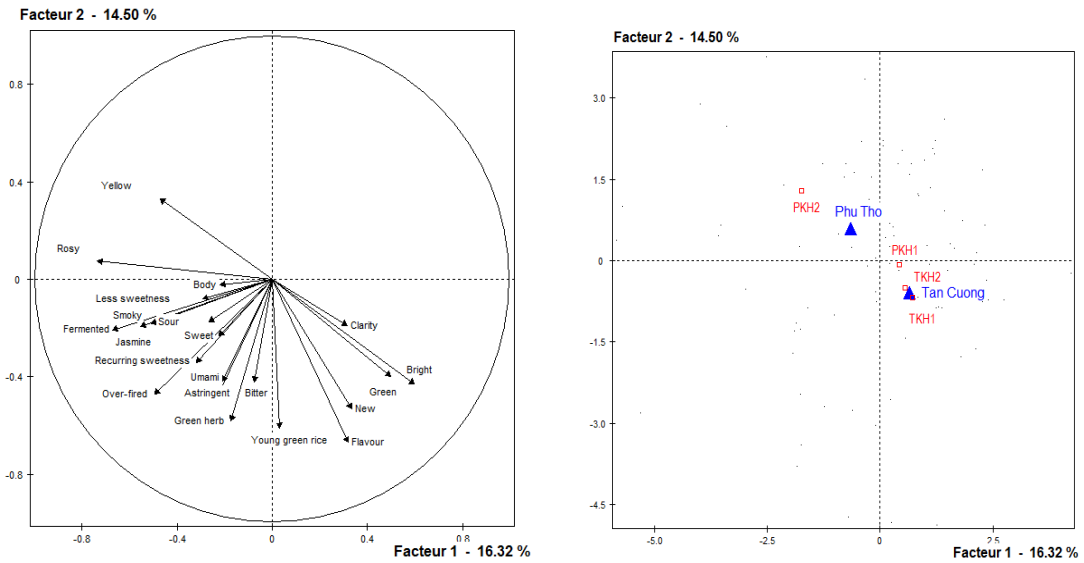

Fig. 3 PCA analysis on brewed tea. Left: correlation of the attributes with the first two components of the PCAs; right: projection of the products onto the first two PCs.

leads us to believe that Phu Tho tea also has a good production procedure that makes a similar quality to Tan Cuong products in terms of color and appearance.

3.3 Brewed Leafs

Similarly, we did an analysis of brewed leaf on the attributes coppery, soft, bright, green, grade. Table 7 shows the results of Tukey test on attributes of brewed leaf.

Results show that Tan Cuong tea has a lower intensity of copper color, leaves are smaller however they have a higher intensity of bright, green and soft in compare to Phu Tho tea. This result is related to previous results of analysis on dry tea.

3.4 Synthetic Analysis

To have an overview on the product space, a PCA on all 37 attributes (dry tea, tea infution and infused leaf) was performed; results of this PCA are presented in Fig. 4.

The results show that products from each region were perceived as different by our trained panel. Using the same harvesting method (one bud and two leaves), Tan Cuong teas are characterized by more favourable attributes than Phu Ho, evaluated by the standard of green tea of Vietnam (TCVN 3218:1993). The differences between four products from two separate regions have been shown. However, larger experiments, with a greater number of products, are needed in order to more clearly affirm our results about regional differences.

4. Conclusions and Recommendations

A description on three categories of sensory properties of green tea (dry tea, brewed tea and brewed leaf) shows a perceptive difference between products

Table 7 Average values of attributes of brewed leaf.

No.	Attribute	TKH1 (A)	TKH2 (B)	PKH1 (C)	PKH2 (D)	*P*-value	Order of evaluation
1	Green	6.9^{c}	6.5^{c}	5.1^{b}	3.1^{a}	0.000	DCBA*
2	Coppery	1.5^{a}	1.8^{a}	3.3^{b}	4.5^{c}	0.000	ABCD*
3	Bright	6.1^{b}	5.4^{b}	5.2^{ab}	3.9^{a}	0.001	DCBA*
4	Grade	4.2^{b}	2.7^{a}	7.2^{c}	6.9^{c}	0.000	BADC*
5	Soft	6.7^{b}	6.9^{b}	3.8^{a}	2.9^{a}	0.000	DCAB*

[abc] the same letter belongs to the same group.

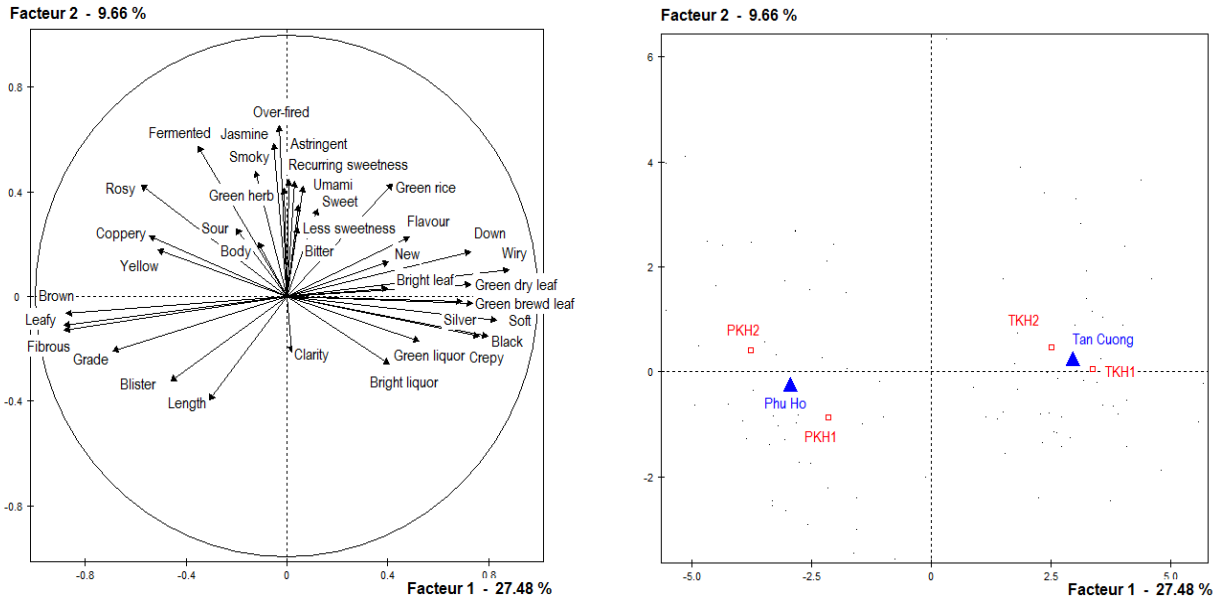

Fig. 1 Synthetic PCA analysis on dry tea, brewed tea and brewed leaf. Left: correlation of the attributes with the first two components of the PCAs; right: projection of the products onto the first two PCs.

from Tan Cuong, Thai Nguyen and Phu Ho, Phu Tho.

For dry tea and brewed leaf, almost all attributes express the differences between the two regions. For tea infusion, Tan Cuong and Phu Ho products are discriminated by sour, fermented, new, flavour, rosy-like and young green rice. Tan Cuong tea was evaluated to have a better flavour. The transportation of fresh tea leaf in Tan Cuong is better, resulting in the fact that this tea is less fermented. In terms of processing, Tan Cuong has a specific method, which uses higher firing in order make a stronger scent of young green rice but weakening the scent of rose in comparison to Phu Ho tea.

In conclusion, the study shows the difference in processing between the two regions, but those inherent differences were not shown fundamentally.

Further sensory evaluation of these products by professional quality experts needs to be carried out in order to characterize better products from different regions.

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